

THALASSAEMIA

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THALASSAEMIA was first recognized as a separate disease entity by Cooley (Cooley and Lee, 1925; Cooley, 1927). Since that time a number of related disorders have been described and these have been classified under the same general title. Because the main feature of these diseases was anaemia, associated with a deficiency of haemoglobin in the red cells, the primary abnormality has long been thought to be impaired haemoglobin synthesis. At first, the disease was classified in three groups: thalassaemia major, thalassaemia minor and thalassaemia intermedia according to the severity of the disease. Although it was soon found that there was a genetic relationship between the various types, nothing was known of the underlying abnormality.

Recent advances in the understanding of the structure, synthesis and genetic control of haemoglobins have led to a greater knowledge of the underlying abnormality in thalassaemia. Although the primary cause is still subject to speculation, the present concepts on the causation of the disease are leading to hypotheses which are directly open to experimental test. Furthermore, the classification of these diseases is of help in determining the prognosis in individual cases, as well as enabling one to give assistance by genetic counselling.

Recently, several reviews on the subject of thalassaemia have appeared (Fessas, 1964; Motulsky, 1964a). There have also been a conference (Fink, 1964), and two monographs (Bannerman, 1961; Weatherall, 1965) devoted entirely to this disease.

Normal Human Haemoglobins

In order to understand the present concepts of the aetiology of the disease, it is necessary to have a clear picture of the genetic control of human haemoglobin synthesis. This area has been reviewed in detail elsewhere (for references see Schroeder, 1963; Huehns and Shooter, 1965).

Adult Haemoglobins

During adult life, the main pigment found

in the red cells is called adult haemoglobin, or Hb-A. Each molecule of Hb-A consists of four globin (polypeptide)-chains arranged in two pairs, two so-called α -chains and two β -chains. To each of these chains is attached a haem group. The structure of Hb-A can thus be written Hb- $\alpha_2\beta_2$ (Schroeder, 1963).

A second adult haemoglobin, called haemoglobin A₂ (Hb-A₂) is also found in the red cell. In the normal person, this amounts to 1.5-3% of total haemoglobin (Kunkel, Ceppellini, Muller-Eberhard and Wolf, 1957; Silvestroni, Bianco, Muzzolini, Modiano, Vallesneri, 1957). This minor haemoglobin has a very similar structure to that of Hb-A, being composed of two α -chains and two δ -chains, and can thus be written Hb- $\alpha_2\delta_2$ (Ingram and Stretton, 1962a and b).

A trace of foetal haemoglobin (Hb-F) (less than 0.4%) (see below) is also found in the red cells of adults (Chernoff, 1953; Beaven, Ellis and White, 1960). Because it is very difficult to measure such small amounts accurately, the upper level of normal is taken as 1% when measured by the method of Betke, Marti and Schlicht (1959) or 2% when measured by the method of Singer, Chernoff and Singer (1951). Using starch gel electrophoresis with a tris-EDTA-borate buffer, pH 8.5, increases of between 1 and 2% of Hb-F can be detected (Huehns and Shooter, 1965; Chernoff, Pettit and Northrop, 1965).

Foetal Haemoglobin

During foetal life, the main pigment present in the red cells is foetal haemoglobin, or Hb-F. This consists of two α -chains joined to two γ -chains, Hb- $\alpha_2\gamma_2$ (Schroeder, 1963).

It should be noted that the three normal haemoglobins all contain the same α -chains and their own type of non- α -chains: β -chains in Hb-A, δ -chains in Hb-A₂ and γ -chains in Hb-F.

In normal cord blood, ca 80% of the pigment found is Hb-F; almost all the rest is Hb-A. A trace of Hb-A₂ can also be detected by

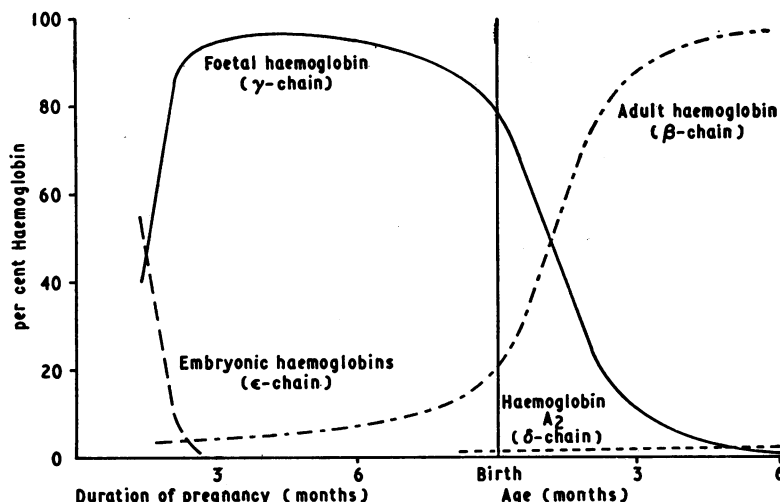


FIG. 1.—Developmental changes in human haemoglobins (taken from Huehns, E. R., and Shooter, E. M. (1965), *J. Med. Genet.*, 2, 48, with kind permission of the Editor and the B.M.A.).

starch gel electrophoresis. This method also shows the presence of small amounts of Hb- γ_4 (Fessas and Mastrokalos, 1959; Karaklis and Fessas, 1963; Weatherall, 1963; Huehns, Dance, Beaven, Hecht and Motulsky, 1964a). Hb- γ_4 has previously been called "fast foetal" haemoglobin (Fessas and Papaspyrou, 1957) or "Bart's" haemoglobin (Ager and Lehmann, 1958). That this haemoglobin consists of four γ -chains (the non- α -chain of foetal haemoglobin) was established by Hunt and Lehmann (1959) and Kekwick and Lehmann (1960). In normal cord blood samples the amount of Hb- γ_4 does not exceed 0.5% (Huehns, Beaven and Stevens, 1964).

During embryonic life, two further normal haemoglobins occur: embryonic haemoglobin (Gower) 1 and embryonic haemoglobin (Gower) 2 (Huehns, Flynn, Butler and Beaven, 1961; Huehns and others, 1964a). These haemoglobins also contain their own specific non- α -chain, the ϵ -chain. The developmental changes in haemoglobin found in the red cells are given in Figure 1. The relative electrophoretic mobilities of the normal human haemoglobins are shown in Figure 2a.

The Genetic Control of Haemoglobin Synthesis

From the study of the abnormal haemoglobins it has been shown that the structure of each of the haemoglobin chains is controlled by a separate genetic locus and that each

gene at each locus finds expression in its own messenger RNA "template", which, in turn, determines the synthesis of a particular polypeptide chain. The various polypeptide chains synthesized then associate with each other and their haem groups to form the haemoglobins found *in vivo* (Figure 2). It has been suggested that there is only one structural locus for the α -chains of Hb-A, Hb-F and Hb-A₂. That this is so is confirmed by the finding that any abnormality of the α -chain affects Hb-A₂ and Hb-F as well as Hb-A (see Huehns and Shooter, 1965, for references).

As has already been noted, the synthesis of embryonic haemoglobins is followed by the synthesis of foetal haemoglobin, which, in turn, is replaced by the adult haemoglobins, Hb-A and Hb-A₂. It has been suggested that these changes are brought about by a number of regulatory genes which control the rates of synthesis of the various polypeptide chains (Neel, 1961; Motulsky, 1962; Zuckerkandl, 1964).

The Haem Groups

The haem groups of the different human haemoglobins are all identical, ferrous protoporphyrin IX. These are synthesized separately from the globin chains by a complex series of enzymic reactions (Rimington, 1959). The genetic control of these processes is separate from that of the protein part of the molecule.

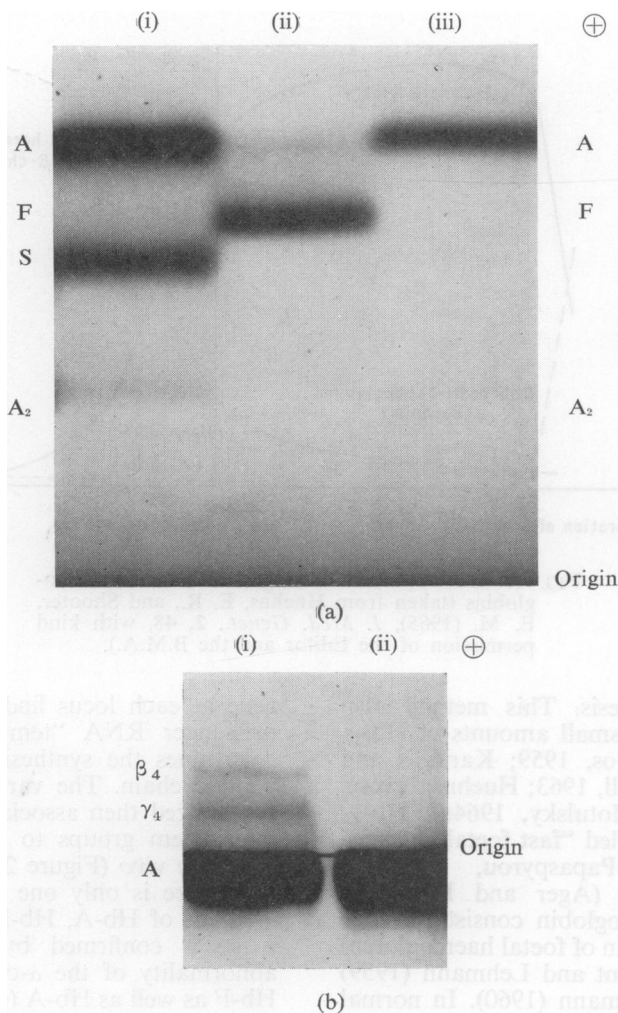


FIG. 2.—Starch gel electrophoresis of human haemoglobins.

(a) *Tris*-EDTA-borate buffer, pH 8.6:

- (i) sickle cell trait showing Hb-A, Hb-S and Hb-A₂
- (ii) normal cord blood showing Hb-A and Hb-F
- (iii) normal adult blood showing Hb-A and Hb-A₂.

(b) Phosphate buffer, pH 7.0:

- (i) Hb-H disease showing Hb-A, Hb-γ₄ and Hb-β₄ (taken from Huehns *et al.*, (1960), *Brit. J. Haemat.*, 6, 388, with kind permission of the Editor and Blackwell Scientific Publications).
- (ii) normal adult.

THALASSAEMIA

The characteristic abnormality in thalassaemia is the reduction in the amount of haemoglobin in the red cells due to diminished haemoglobin synthesis. As haemoglobin consists of the haem group joined to the protein moiety, globin, impairment of haemoglobin synthesis could be due to diminished synthesis of either haem or globin.

The finding that there was impairment in the incorporation of ^{14}C -glycine into haem and some haem precursors in *in vitro* bone marrow cultures prepared from patients with thalassaemia (Bannerman, Grinstein and Moore, 1959; Steiner, Baldini and Damashek, 1964) appears to support the idea that the abnormality in this disease is one of haem synthesis. Further support for this comes from the observation that in thalassaemia there is a great increase in the excretion of early labelled bile pigment after the injection of ^{15}N or ^{14}C -l-glycine (Grinstein, Bannerman, Vavra and Moore, 1960). On the other hand, the well-documented clinical observation that infants with thalassaemia major only develop the disease during the first year of life is opposed to the idea that the primary abnormality is a defect in haem synthesis. This is because the haem groups of foetal haemoglobin and adult haemoglobins are identical, namely, ferrous protoporphyrin IX, and hence impairment of its synthesis should lead to anaemia during foetal as well as adult life. On the other hand, the protein parts of these molecules differ, containing γ -chains in foetal haemoglobin ($\text{Hb-}\alpha_2\gamma_2$) and β -chains in adult haemoglobin ($\text{Hb-}\alpha_2\beta_2$). Recent work has shown that haem exerts a negative feedback on the synthesis of δ -amino-laevulinic acid (ALA) in red cell precursors (Lascelles, 1960; Burnham and Lascelles, 1964; London, Bruns and Karibian, 1964; Steiner and others, 1964). The reduced rate of haem synthesis in thalassaemia can thus be explained by "feedback" inhibition of the synthesis of δ -ALA by the un-utilized haem formed in the thalassaemic red cell. The abnormality in bile-pigment labelling mentioned above is probably due to the large amount of "ineffective erythropoiesis" (see later) found in this disease, rather than to an abnormality of haem synthesis. It would therefore appear more likely that thalassaemia is due to an abnormality of globin synthesis. This has recently been confirmed by the work of Marks and Burka (1964) who have shown that the synthesis of β -chains is impaired in the "cell-free system",

using ribosomes obtained from β -thalassaemic red cell precursors.

As has already been pointed out, the various globin chains (α -, β -, δ -, γ - and ϵ -chains) are separately synthesized, and any abnormality of globin synthesis would thus be expected to affect primarily only one type of chain. This implies that just as there are two main groups of abnormal haemoglobins, those affecting the α -chains and those affecting the β -chains, there would also be two groups of thalassaemias, one affecting the synthesis of α -chains and one affecting the synthesis of β -chains, and these have been called α -thalassaemia and β -thalassaemia respectively. The finding of rare individuals without any Hb-A_2 (Fessas and Stamatoyannopoulos, 1962; Warrington, Bell and Thompson, 1965) suggests that there might also be a mutation specifically inhibiting δ -chain synthesis, δ -thalassaemia. However, the absence of Hb-A_2 on electrophoresis could also be explained if these individuals were the carriers of a δ -chain mutation which resulted in a Hb-A_2 variant migrating with Hb-A (Baglioni, 1964). The existence of γ -thalassaemia has also been claimed (Hamilton, Sheets and Brosseau, 1962). However, the only two established groups are α - and β -thalassaemia.

The genes causing thalassaemia can, of course, occur in the heterozygous or homozygous state. There would thus be α - and β -thalassaemia trait and α - and β -homozygous thalassaemia. The action of the abnormal genes results in a reduction in the rate of synthesis of the particular polypeptide chain involved. The heterogeneity of clinical severity of the disease suggests that there are a number of different mutations causing thalassaemia. It is thought that these differ in the degree of impairment of polypeptide chain synthesis caused. Some mutations would completely, or almost completely, inhibit the synthesis of a particular polypeptide chain, while others would only partially inhibit it. The former may be called "severe" thalassaemia genes and the latter "mild" thalassaemia genes.

In the simple thalassaemia heterozygote, it is difficult to measure the degree of inhibition caused by any particular thalassaemia gene. However, an approximate measure of this is obtained from the proportion of Hb-A found in subjects who also carry an abnormal haemoglobin. This is illustrated in mixed heterozygotes for β -thalassaemia and Hb-S or Hb-C (for references see later). If no Hb-A is found, it may be inferred that the complete suppression of β -chains has been caused by the thalas-

TABLE 1
CLINICAL FEATURES OF THALASSAEMIA
(modified from Pearson, 1964)

	Major	Intermedia	Minor
Haemoglobin (g./100 ml.)	<6	6 - 10	>10
Reticulocytes (%)	2-15	2-10	<5
Nucleated red cells	++++ particularly following	+++ to 0 splenectomy	0
Abnormal red cell morphology	++++	+++	+
Jaundice	++	+0	0
Splenomegaly	+++	++	±
Skeletal changes	+++ to ++	+++ to 0	0
Transfusion requirement	+++ to +	+ to 0	0

saemia gene. In others, various amounts of Hb-A are found, due to different degrees of inhibition of β -chain synthesis. The same argument applies to α -thalassaemia interacting with haemoglobins with abnormal α -chains.

Clinical Features

The clinical features of the thalassaemias have been described by a number of authors (Astaldi, Tolentino, Sacchetti, 1951; Dacie, 1960; Wintrobe, 1963); only the main features will be outlined here and are summarized in Table 1. As the diseases caused by deficient α - or β -chain synthesis are very similar, it is convenient to give a general description first. The specific features of each type will be discussed later.

Thalassaemia Major

This is a severe anaemia with a haemoglobin of 3-6 g./100 ml. There is gross enlargement of the spleen and liver, partly due to infiltration with erythropoietic tissue. Besides extramedullary erythropoiesis, there is gross expansion of the red marrow. This leads to typical X-ray appearances of the skeleton (Cooley, Witwe and Lee, 1927; Caffey, 1957; Mosely, 1962; Baker, 1964). In the most severe cases, even the small bones of the hands and feet are affected (Fig. 4a). Pathological fractures are not uncommon. The skull shows a distinctive

appearance (Fig. 4b.). There is atrophy of the outer table, with a radiating growth of subperiosteal bone, giving the so called "hair on end" appearance. The facial bones are also expanded, giving the typical mongoloid facies.

The mean red cell survival is only moderately shortened to approximately 10-80 days, but there is also a small proportion of cells which are destroyed very rapidly (Kaplan and Zuelzer, 1950; Sturgeon and Finch, 1957; Erlandson, Schulman, Stern and Smith, 1958; Bailey and Prankerd, 1958). There is clearly a discrepancy between red cell productive capacity and the rate of red cell production. Sturgeon and Finch (1957) have shown that the rate of ^{59}Fe uptake by the marrow greatly exceeds that appearing in the red cells. They argue that a large proportion of the red cells produced in the marrow are not released into the circulation but destroyed *in situ*, a process called "ineffective erythropoiesis".

Haematology. The total haemoglobin is extremely low: 3-6 g./100 ml. The red cells show gross variation of size and shape with marked hypochromia and target cells (Fig. 5). The MCH and MCHC are both low. Nucleated red cells are prominent in the blood film, particularly after splenectomy. The cells are extremely thin, the mean cell average thickness (MCAT) being *ca* 1.0-1.5 μ compared with a normal of 2.2 μ . This thinness of the cells is reflected in their extreme resistance to osmotic

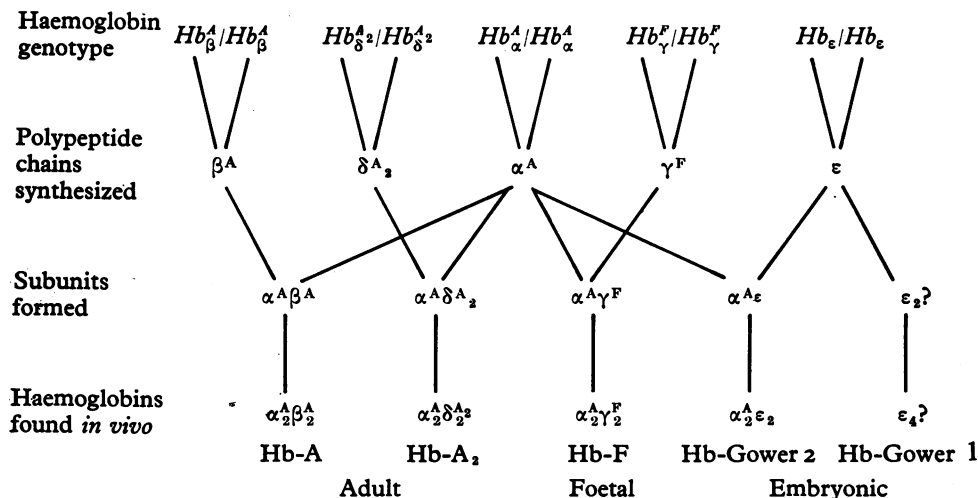


FIG. 3.—Genetic control of the synthesis of normal human haemoglobins (from Huehns and Shooter, 1965).

lysis. Occasionally, some cells are not even lysed by water. The plasma contains a raised bilirubin and also some methaemalbumen. As in other haemolytic diseases, the iron-binding capacity of the plasma is fully saturated. The bone marrow shows extreme hyperplasia of the erythropoietic cells, usually of the normoblastic type. Occasional cases with megaloblastic changes are seen (Chanarin, Dacie and Molin, 1959).

Thalassaemia Minor

This is a mild disease, there being no, or only borderline, anaemia. The red cells show moderate variation in size and shape with some hypochromia. Target cells are often found. There is increased resistance of the red cells to osmotic lysis. The most characteristic finding is a low MCH and a reduction in the MCV. Occasionally the spleen may be palpable. There are no X-ray changes in the bones. The mean red cell survival is normal (Kaplan and Zuelzer, 1950; Malamos, Belcher, Gyftaki and Binopoulov, 1961). The clinical picture is often difficult to distinguish from that of iron deficiency anaemia.

Thalassaemia Intermedia

In this syndrome, the haemoglobin varies from *ca* 6 to 10 g./100 ml. The spleen is usually palpable and may be very large. In many patients there is also liver enlargement. The expansion of the bone marrow varies from patient to patient, depending on the severity of the disease. Thus, in the more severely affected gross X-ray changes in the bones are found,

whilst in milder cases small or no changes may be detectable. The blood picture is typical of thalassaemia.

Classification of Thalassaemia

(i) *β-thalassaemia*

In *β-thalassaemia*, the rate of synthesis of the *β*-chains is suppressed whilst that of the other polypeptide chains is not primarily affected. It can be seen from Figure 1 that at birth the *γ*-chains of foetal haemoglobin are dominant, and no disease would be expected at this stage of development but would commence at *ca* three months of age. Any deficiency of *β*-chains would lead to a relative increase in *γ*- or *δ*-chains if these are produced at a normal rate. The *γ*-chains might also compensate for the *β*-chain deficiency if they are produced in quantity after the neonatal period (see Fig. 3). The specific features of *β-thalassaemia* are, therefore, commencement of the disease after birth, together with a relative increase in Hb-A₂ and/or Hb-F in the red cells.

(a) *β-thalassaemia trait*. The typical condition, sometimes called classical thalassaemia trait, is characterized by a relative increase in Hb-A₂* above the normal level of 1.5-3% (Kunkel and Wallenius, 1955; Kunkel and others, 1957; Gerald and Diamond, 1958a; see also Weatherall, 1965). For example, Silvestroni and Bianco (1963) give their range for normal

*There are two reports of a raised Hb-A₂ other than in thalassaemia: pernicious anaemia (Josephson, Masri, Singer, Dworkin and Singer, 1958) and after bone marrow transplantation (Bridges, Neill and Lehmann, 1961).

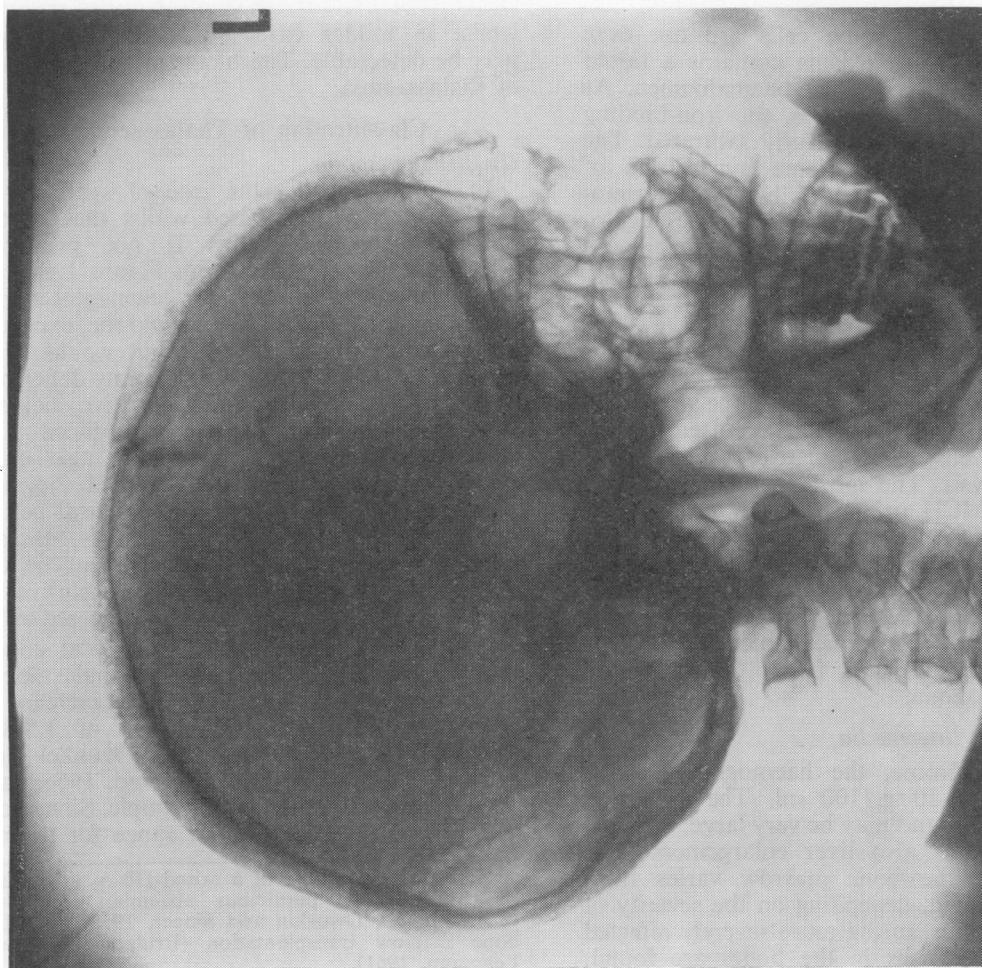


FIG. 4.—X-ray changes in thalassaemia major.

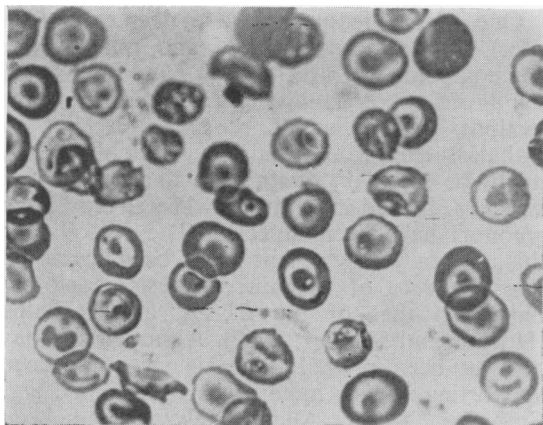
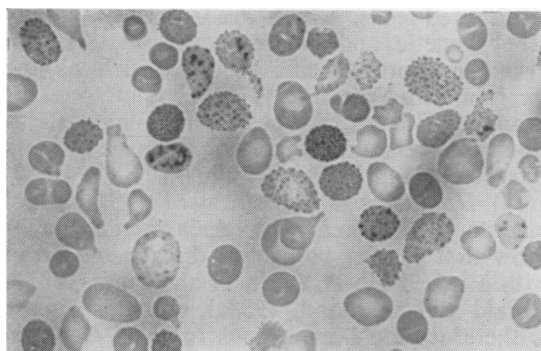


FIG. 5.—(a) Red cell changes in thalassaemia intermedia following splenectomy.



(b) Inclusion bodies in the red cells of Hb-H disease following incubation with brilliant cresyl blue, (photograph by courtesy of Dr. J. C. White).

values as $1.95 \pm 2\sigma$ 1.3-2.6 whilst that for β -thalassaemia trait was $4.22 \pm 2\sigma$ 3.2-5.3. Only occasional cases with Hb-A₂ levels greater than 6% are found (Kunkel and others, 1957; Carcassi, Ceppellini and Pitzus, 1957; Josephson, Masri, Singer, Dworkin and Singer, 1958; and Weatherall, 1964a). About half these patients also have a raised Hb-F; this is usually in the 1-5% range with a mean level of 2.1% (Beaven, Ellis and White, 1961; Weatherall, 1964a). Occasionally, higher levels are found (see Weatherall, 1965). In the post-natal period, the rate of disappearance of Hb-F in β -thalassaemia trait is significantly slower than in normal infants (Beaven, Ellis and White, 1961), and a raised Hb-F is an almost consistent feature of the disease in young children. The Hb-F found in this condition is unevenly distributed in the red cells (Shepard, Weatherall and Conley, 1962). Clinically the condition is a form of thalassaemia minor and usually the blood films show typical changes. However, in occasional patients the only finding is a raised Hb-A₂, the red cells being otherwise normal (Gouttas, 1962; Silvestroni and Bianco, 1957; Carcassi and others, 1957).

There is another group of patients with the blood picture of thalassaemia minor and a raised Hb-F, but with a normal Hb-A₂ (Zuelzer, Robinson and Booker, 1961; Fessas, 1962b; Gabudza, Nathan and Gardner, 1964; Weatherall, 1964a). These patients may have Hb-F

levels higher than in classical β -thalassaemia trait. This form of β -thalassaemia trait also interacts with the classical type of β -thalassaemia trait with a high Hb-A₂ to give the picture of β -thalassaemia major or intermedia. The explanation for the failure to find an increase of Hb-A₂ in these cases is not clear. Some patients may be suffering from concomitant iron deficiency, which is known to reduce the level of Hb-A₂ (Josephson and others, 1958; Chernoff, 1964). However, this does not explain all cases. Some authors have suggested that the mutation not only affects the synthesis of the β -chains but also that of the δ -chains (Bernini, Colucci, De Michele, Piomelli, and Siniscalco, 1962; Fessas, 1964; Motulsky, 1964a), and the latter authors have coined the term " $\beta\delta$ -thalassaemia". In other patients, the failure to find a raised Hb-A₂ might be caused by the inheritance of a β -thalassaemia gene which only partially suppresses β -chain synthesis.

Occasionally, the clinical picture of thalassaemia minor is due to the presence of an abnormal haemoglobin with a low rate of synthesis, such as Hb-Lepore (see later) or Hb-Caserta (Ventruto, Baglioni, Rosa, Bianchi, Colombo and Quattrin, 1965).

From the above description it will be seen that the phenotypic expression of β -thalassaemia trait is very variable. In most families, the expression of the gene in various members is reasonably constant, whilst in others there is considerable variation, not only affecting the

degree of anaemia and morphological changes in the red cells, which are subject to many other genetic and environmental factors, but also the more specific features such as the level of Hb-A₂ and of Hb-F found (see, for example, Beaven, Stevens, Ellis, White, Bernstock, Masters and Stapleton, 1964; Lee, Haut, Cartwright and Wintrobe, 1964).

(b) *Homozygous β -thalassaemia*. Studies of parents of children with thalassaemia major show that usually both have thalassaemia trait with a raised Hb-A₂ (Kunkel and others, 1957; Gerald and Diamond, 1958a), and this disease is therefore the homozygous form of β -thalassaemia. Because β -chain synthesis is (almost) completely inhibited, a severe disease results which begins at the time in development when γ -chain synthesis normally ceases and β -chain synthesis commences. The severe anaemia produces a stress situation in the erythroid marrow, which, by a mechanism not yet understood, attempts to compensate for the failure of β -chain synthesis by the continued production of γ -chains. However, the rate at which these are produced is far below that necessary for complete compensation.*

It appears that the Hb-F produced by such a situation is unevenly distributed among the red cell population. This has been shown in sickle cell disease (Singer and Fisher, 1952) and β -thalassaemia minor (Shepard and others, 1962). As Hb-F is the only pigment produced in quantity in β -thalassaemia major, this variation in the amount of Hb-F synthesized leads to cells containing varying amounts of haemoglobin. Those cells which contain very little haemoglobin are destroyed in the marrow, the so called "ineffective erythropoiesis" already referred to. The remaining, relatively few, cells reach the circulation. Some of these cells are rapidly destroyed, whilst the remainder have a moderately shortened survival. (For references see earlier). Gabudza, Nathan and Gardner (1963) found that the short-lived cells contain relatively less Hb-F and, on the above hypothesis, would contain less total haemoglobin than the remainder. The red cells in the circulation contain mostly Hb-F. This usually forms 60% or more of the total haemoglobin, but levels from as low as 10% up to 100% have been reported (see Weatherall, 1965). Hb-A₂ is usually within normal limits (Kunkel and others, 1957; Silvestroni and others, 1957). The remainder is Hb-A. A trace of Hb- α is also present (Fessas and Loukopoulos, 1964).

*The situation in which complete compensation occurs is called hereditary persistence of foetal haemoglobin, referred to later, and does not lead to anaemia.

One of the features of α -thalassaemia (see later) is the occurrence of the non- α -chain haemoglobins, Hb- β_4 , Hb- γ_4 and Hb- δ in significant amounts in the red cells (see next section), and it might be expected that in β -thalassaemia free α -chain haemoglobin, Hb- α , might be found in *quantity* in the red cells of severely affected individuals, whereas only trace amounts have been detected. The reasons for this are not yet clear, but it has been suggested that the rate of α -chain synthesis is closely linked to that of the β -, γ -, and δ -chains (Huehns and Shooter, 1962). Although this has not yet been proved, there is some evidence supporting this hypothesis (Baglioni and Colombo, 1964). Alternatively it has been suggested that the excess α -chains formed precipitate in the red cells (Fessas, 1963).

One of the puzzling features of β -thalassaemia is that though the proportion of Hb-A₂ is raised in β -thalassaemia trait, it is usually normal or low in the homozygous state. If δ -chain synthesis were proceeding at a normal rate, it might be expected that Hb-A₂ would form a much higher proportion of total haemoglobin in the homozygous condition than in the trait form. A possible explanation is that the continued synthesis of γ -chains suppresses that of the δ -chains by some kind of "feedback" mechanism.

(c) *β -thalassaemia intermedia*. There are many cases which do not fit the clinical picture of β -thalassaemia major or minor. These are called β -thalassaemia intermedia. Hb-F forms from 10-80% of total haemoglobin in the red cells, whilst Hb-A₂ is usually raised above normal and in some cases is above 6% (Kunkel and others, 1957; Josephson and others, 1958; Gabudza and others, 1963; Pearson, 1964).

The exact genetic causation of these syndromes is not clear, but they may represent the interaction of two β -thalassaemia genes, one of which is of the severe type, causing (almost) complete suppression of β -chain synthesis, whilst the other causes only partial suppression. Studies of the parents of these patients have shown that in some families both have thalassaemia trait with a raised Hb-A₂ (Pearson, 1964; Weatherall, 1965), whilst in others only one parent shows this type (Zuelzer and others, 1961; Fessas, 1962; Wolf and Ignatov, 1963; Gabudza, Nathan and Gardner, 1964).

(d) *The interaction of β -thalassaemia with a β -chain variant of haemoglobin A*. β -thalassaemia only interacts with haemoglobin variants with abnormal β -chains. The result of such

interaction is the reduction in the number of β^A -chains synthesized, leading to an alteration in the ratio of Hb-A to abnormal haemoglobin found when compared to that present in the simple heterozygous haemoglobinopathy. If a β -thalassaemia gene which completely suppresses β^A -chain synthesis is inherited, no Hb-A will be found. With other genes, various proportions of Hb-A are found. The ratio of Hb-A to abnormal haemoglobin appears constant in any one family (Fessas, 1964).

The most common of these syndromes is sickle cell-thalassaemia disease. This disease has been recognized for a long time and is described in detail by Singer, Singer and Goldberg (1955). Clinically, it is a form of sickle cell disease rather than thalassaemia. The severity varies considerably, and it is often difficult to distinguish homozygous sickle cell disease from sickle cell-thalassaemia disease. A definite diagnosis can, of course, be made by family studies. Hb-F levels do not distinguish between the two conditions, the range of values being very similar [homozygous sickle cell disease from 2-18% (Huisman and Dozy, 1962) and 3-32% (Beaven and others, 1961); sickle cell- β -thalassaemia disease from 1-20% (Beaven and others, 1961; Monti, Feldhake and Schwartz, 1964; see also Weatherall, 1965)]. Hb-A₂ levels appear to distinguish between the two conditions, although the available data are very scanty. In homozygous sickle cell disease the Hb-A₂ level is usually normal, whilst in sickle cell-thalassaemia it is usually raised, but some overlap does occur. In homozygous sickle cell disease the range for Hb-A₂ reported is 1.7-4.6%, with 21 cases within the normal range of 1.5-3% and 2 above (Huisman and Dozy, 1962; Aksoy, 1963); in sickle cell- β -thalassaemia 16 cases examined, range 2.4-7.0% (Henderson, Potts and Burgess, 1962; Aksoy, 1963; Monti and others, 1964).

Other syndromes of this type are Hb-C-thalassaemia disease (Singer, Kraus, Singer, Rubenstein and Goldberg, 1954; Zuelzer and Kaplan, 1954), Hb-D-thalassaemia disease (Hynes and Lehmann, 1956), Hb-E-thalassaemia disease (Na-Nakorn and Minnich, 1957; Kochhar and Kathpalia, 1963), and Hb-J-thalassaemia disease (Sydenstrycker, Horton, Payne and Huisman, 1961). Clinically, these are variants of β -thalassaemia of intermediate to mild severity. The diagnosis is made from the clinical picture and the electrophoretic analysis of the haemolysate.

(e) *Haemoglobin Lepore*. Haemoglobin Lepore gives rise to a number of syndromes

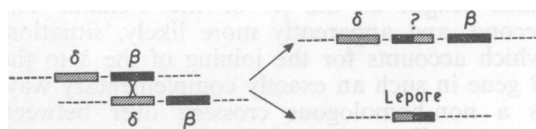


FIG. 6.—Schematic representation of the postulated non-homologous crossing-over leading to the formation of the Hb-Lepore genes (from Baglioni, C. (1962), *Proc. Nat. Acad. Sci. (Wash.)*, **48**, 1880, with permission of the National Academy of Sciences, Washington).

very similar to the true thalassaemias. Furthermore, this haemoglobin is found not infrequently in areas of the world where thalassaemia is commonly found. Haemoglobin Lepore was first described by Gerald and Diamond (1958) in an Italian family and has also been reported from Indonesia (Neeb, Beiboer, Jonxis, Kaars Sijpesteijn and Muller, 1961). Several other reports of a similar haemoglobin have appeared in the literature: "Hb-Pylos" was found in Greece (Fessas, Stamatoyannopoulos and Karaklis, 1962) and "Hb-G"* in Italy (Silvestroni and Bianco, 1958), and these are probably other examples of this haemoglobin. Lepore haemoglobin migrates with Hb-S on starch block or gel electrophoresis.

Structural studies of these haemoglobins show that they are composed of two normal α -chains joined to two non- α -chains; these non- α -chains consist of the same number of amino acids as the β -chains, but the amino acid sequence is like that of the δ -chain at the N-terminal end and the β -chain at the C-terminal end (Baglioni, 1962); they have therefore been called $\delta\beta$ -chains. In haemoglobin Lepore Boston (Baglioni, 1965) the $\delta\beta$ -chain consists of δ -chain sequence from the N-terminal amino acid to *ca* amino acid no. 87 and of β -chain sequence from *ca* no. 116 to the C-terminal end. The amino acids in the region 88-115 could belong to either the δ - or β -chain. In Hb-Lepore Hollandia amino acids 1-22 have the δ -chain sequence, 50-146 the β -chain sequence and 23-49 could have arisen from either chain (Barnabas and Muller, 1962).

Baglioni (1962) has discussed the genetic event that could have led to the formation of a $\delta\beta$ -polypeptide chain. Two possibilities are mentioned, both of which require the Hb β and Hb δ loci to be closely linked (see later). The first possibility is a deletion of part of the δ and β loci with the formation of a new $\delta\beta$ gene. On this hypothesis it is difficult to understand why the $\delta\beta$ -chain synthesized is the

*Most haemoglobin variants designated "Hb-G" have the usual single amino-acid substitution.

same length as the β - or the δ -chain. The second, and apparently more likely, situation, which accounts for the joining of the δ to the β gene in such an exactly complementary way, is a non-homologous crossing over between corresponding points of the β and the δ genes, resulting in the formation of unequal products, one of which is Hb-Lepore (Figure 6).

Clinical Features

Hb-Lepore trait (Gerald and Diamond, 1958b; Neeb and others, 1961; Fessas and others, 1962). Individuals carrying this trait have a blood picture similar to that found in thalassaemia trait. Starch gel or starch block electrophoresis reveals the abnormal haemoglobin, which amounts to 10-15% of total haemoglobin. Hb-A₂ is within normal limits, and Hb-F may be normal or slightly raised.

Homozygous Lepore disease. (Neeb and others, 1961; Fessas and others, 1962). Clinically, this condition is a severe type of thalassaemia intermedia. No Hb-A or Hb-A₂ is found; Hb-Lepore amounts to 12% of total haemoglobin, the remainder being Hb-F.

Hb-Lepore- β -thalassaemia disease (Gerald and Diamond, 1958b; Pearson, Gerald and Diamond, 1959; Fessas and others, 1962) is a severe form of β -thalassaemia intermedia. Most of the haemoglobin in the red cells is Hb-F; Hb-A₂ is low or normal in amount and Hb-Lepore forms 5-10% of total haemoglobin. No Hb-A is found.

(ii) *The interaction of α -with β -thalassaemia* (Fessas, 1962).

Clinically, this disease is a form of thalassaemia minor. The diagnosis can only be established by family studies.

(iii) *α -thalassaemia*

As the α -chains of Hb-A, Hb-F and Hb-A₂ are identical, α -thalassaemia affects haemoglobin production both before birth as well as later in life, and it appears that the effect is clinically more severe during foetal life than later.

(a) *α -thalassaemia trait.* Clinically, this condition is even milder than β -thalassaemia trait. Presumably, this is because the remaining normal Hb α gene is able to give almost full compensation. The clinical features of the trait have been investigated by Malamos and others (1962) and Weatherall (1964b). At birth, these individuals carry 5-10% Hb- γ_4 , and their red cells already show the typical morphology of thalassaemia minor. During the neonatal period, this disappears, together with Hb-F (Ager and Lehmann, 1958). Later in life, the patient has slight anaemia with a blood film suggesting

thalassaemia. The Hb-A₂ level is normal. It is, therefore, clearly difficult to distinguish these cases from $\beta\delta$ -thalassaemia mentioned earlier or from mild iron deficiency. (The latter can be excluded by estimating the serum iron. Occasionally, iron deficiency and thalassaemia minor occur in the same patient. The blood picture in these is more severe than in the simple trait). The finding of Hb- β_4 or Hb- γ_4 in any relatives helps in the diagnosis. Malamos, Fessas and Stamatoyannopoulos (1962) have described the presence of occasional red cells with inclusion bodies after incubation with brilliant cresyl blue, presumably due to the presence of Hb- β_4 . In a few patients, a trace of Hb- γ_4 is found in the haemolysate. The above description of α -thalassaemia trait presumably applies to a gene which causes complete suppression of α -chain synthesis, since in individuals in whom there is interaction of an α -thalassaemia gene of this type with an α -chain mutation of haemoglobin no normal α^A -chains are formed (Dormandy, Lock and Lehmann, 1961; Lie-Injo Luan Eng and Hart, 1963).

The situation in α -thalassaemia trait which only partially suppresses the synthesis of α -chains is not clear. In adults, this is probably undetectable. At birth, these may have a slight increase in Hb- γ_4 , in the region of 1-2% of total haemoglobin. Such individuals are rare (<1%) in England but are more frequent (ca 12%) in individuals originating overseas (author's unpublished observations). One case has been studied in some detail. This infant was an offspring of a patient with Hb-H disease (Case 2, Dance, Huehns and Beaven, 1963). Total haemoglobin in the cord blood was 17.1 g./100 ml., and the red cells showed considerable anisocytosis but no hypochromia; the serum bilirubin was 2.2 mg./100 ml.

(b) *Homozygous α -thalassaemia.* This is due to the inheritance of two α -thalassaemia genes causing severe α -chain suppression (see above). No α -chains are synthesized, and death *in utero* apparently occurs at approximately 32 weeks' gestation. The foetus shows severe hydrops foetalis, anaemia, and erythroblastosis (Lie-Injo Luan Eng, 1962).

(c) *α -thalassaemia intermedia or haemoglobin H disease.* Haemoglobin H (Hb- β_4) was first described in a Chinese family by Rigas, Koler and Osgood (1955, 1956) and in a Greek family by Goultas, Fessas, Tseverenis and Xefteri (1955). It was shown to consist of four β -chains by Jones, Schroeder, Balog and Vinograd (1959) who suggested that it could be the result of diminished α -chain synthesis. Patients with

Hb-H disease suffer from a mild form of thalassaemia intermedia, with haemoglobin levels of 8-12 g./100 ml. There may be a rough correlation between the severity of the disease and the amount of Hb- β_4 found in the red cells. In our own cases, the most affected (Case 2, Bingle, Huehns and Prankerd, 1958) had a haemoglobin of 10 g./100 ml. with 15% Hb- β_4 , whilst a recent patient (unpublished) had only 2% Hb- β_4 with 12.5 g. haemoglobin/100 ml. The clue to the diagnosis of Hb-H disease is the finding of the characteristic inclusion bodies in the red cells on incubation with brilliant cresyl blue (Fig. 5b). Hb-H is best demonstrated using starch gel electrophoresis in phosphate buffer at pH 7.0 (Huehns, Flynn, Butler and Shooter, 1960) (Fig. 2b). Recent work on Hb-H disease leaves little doubt that it is caused solely by a deficiency in α -chain synthesis, and is therefore a form of α -thalassaemia*. The finding that Hb-H consists of four β^A -chains (Jones and others, 1959; Jones and Schroeder, 1963) and that β^A -chains obtained from Hb-A will also form Hb-H (Huehns, 1962; Huehns and others, 1965) indicates that *in vivo* Hb- β_4 is not due to a structural abnormality of its β -chain or the presence of an abnormal enzyme causing the β -chains to form Hb- β_4 . As the α -chains for the formation of the three normal haemoglobins are derived from a common metabolic pool (see Huehns and Shooter, 1965), α -chain deficiency should lead not only to the formation of Hb- β_4 but to the formation of Hb- γ_4 and Hb- δ in the same individual. This has been demonstrated in four patients with Hb-H disease (Dance and others, 1963). The finding that Hb- β_4 occurs together with Hb- γ_4 has previously been reported in a number of patients (Ramot, Sheba, Fisher, Ager and Lehmann, 1959; Fessas, 1960; Huehns and others, 1960). The possibility that other patients with Hb-H disease may also carry Hb- δ is suggested by the low levels of Hb-A₂ commonly found in the disease (Gerald and Diamond, 1958a; Ramot and others, 1959; Dittman, Haut, Wintrobe and Cartwright, 1960; Koler and Rigas, 1961; Dance and others, 1963), some δ -chains being used to form Hb- δ rather than Hb-A₂.

Two other points need explanation. The first is the finding that Hb- γ_4 can occur without any Hb- β_4 , although plenty of β -chains are being

made (Choremis, Zannos-Mariolea, Ager and Lehmann, 1959). It appears that α -chains combine preferentially with β -chains to form Hb-A rather than with γ -chains to form Hb-F (Huehns and others, 1960; Huehns, Beaven and Stevens, 1964). On this hypothesis, the relative amounts of Hb- γ_4 and Hb- β_4 formed at a given level of α -chain deficiency depend solely on the number of γ -chains available. Thus, when γ -chains are available in quantity and β -chains relatively scarce, as, for example, in foetal life and early infancy or in the case reported by Choremis and co-workers (1959), deficiency in α -chain formation could result in all the β -chains being used up in the formation of Hb-A, while those γ -chains left over after the formation of Hb-F would polymerize to produce Hb- γ_4 . Secondly, if it is true that the formation of Hb- β_4 and Hb- γ_4 are both due to diminished α -chain production, it would be expected that all carriers of Hb- γ_4 at birth would later develop Hb- β_4 . Follow-up studies have shown that this is not so (Ager and Lehmann, 1958; Malamos and others, 1962; Weatherall, 1964b). The explanation for this is not at all clear but could be based on a falling off in the absolute rate of non- α -chain synthesis in the postnatal period (Huehns and others, 1960).

When the published pedigrees of families with Hb-H disease are studied, it appears likely that the disease is caused by the interaction of two genes (Huehns and others, 1960; Koler and Rigas, 1961). Huehns and others (1960) have suggested that these are two α -thalassaemia genes. The occurrence of the "severe" type of α -thalassaemia trait in some relatives is well documented. However, the work of Lie-Injo Luan Eng (1962) indicates that the inheritance of two such genes leads to intrauterine death.

It has therefore been suggested that Hb-H disease is caused by the interaction of a "severe" α -thalassaemia gene (causing complete suppression of α -chain synthesis) with a "mild" α -thalassaemia gene (causing partial suppression of α -chain synthesis) or an α -chain haemoglobin mutation (Huehns, 1962). Analysis of the published family data of Hb-H disease are consistent with this hypothesis (Huehns and Motulsky, unpublished; Wasi, Na-Nakorn and Svingdamrong, 1964). As the incidence of the "severe" α -thalassaemia trait in Greece is about 0.4% (Malamos and others, 1962), the occurrence of families in which Hb-H appears in several generations (Gouttas and others, 1955; Bingle, Huehns and Prankerd, 1958) indicates that the postulated "mild" α -thalassaemia gene must be relatively common. The finding that

*Hb-H also occurs in occasional patients with erythroleukaemia (Bergren and Sturgeon, 1960; White, Ellis, Coleman, Beaven, Gratzler, Shooter and Skinner, 1960; Beaven, Stevens, Dance and White, 1963; Finch and Motulsky, unpublished).

slight increases of Hb- γ_4 occur relatively commonly in non-Caucasian cord bloods examined (see above) lends some support to this hypothesis. Further support would be obtained if all newborns from parents with Hb-H disease carried increased Hb- γ_4 . Six such infants have been reported; five of these carried Hb- γ_4 (Fessas, 1960; Benesh, Ranney, Benesh and Smith, 1961; Huehns, unpublished); one infant did not show any abnormal haemoglobin (Gouttas, Tsevenis and Papaspyrou, cited by Fessas, 1960). It is, however, not known whether the latter case was examined by sufficiently sensitive methods to detect the small increases postulated to occur in some of these. Another such infant did not show any Hb- γ_4 by paper electrophoresis at the age of five weeks (Brain and Vella, 1958).

An alternative genetic explanation is that the Hb-H disease results from the presence of an α -thalassaemia gene with a second gene (replacing the "partial" α -thalassaemia gene postulated above) which segregates independently (Rigas and Koler, 1961; Weatherall, 1965). In favour of this hypothesis is the finding that 7 out of 29 offspring from parents with Hb-H disease also had the disease and that one newborn from these parents did not carry Hb- γ_4 .

(d) *The interaction of α -thalassaemia with an α -chain variant of haemoglobin A.* Hb-Q- α -thalassaemia disease clinically resembles Hb-H disease, except that instead of Hb-A, Hb-Q is found (Vella, Wells, Ager and Lehmann, 1958; Dormandy and others, 1961; Lie-Injo Luan Eng and Hart, 1963). Because no Hb-A is present in these cases, the α -thalassaemia would be of the "severe" type. One patient with Hb-I- α -thalassaemia disease has been described (Atwater, Schwartz, Erslev, Montgomery and Tocantins, 1960). Clinically, the picture was that of thalassaemia. The patient's haemolysate showed approximately 80% Hb-I and 20% Hb-A; Hb-A₂ and Hb-I₂ were also present in trace amounts (Atwater, Huehns and Shooter, 1961). In this case, the α -thalassaemia allowed the formation of significant amounts of Hb-A and was therefore of the "mild" type.

(e) *The occurrence of α -thalassaemia with β -chain variants of haemoglobin A.* The occurrence of α -thalassaemia with Hb-E (Tuchinda, Rucknagel, Minnich, Boonyaprakos, Balankura and Savatee, 1964), Hb-C and Hb-S (Zuelzer, Neel and Robinson, 1956; Weatherall, 1964b) has been described. In each case, the relative proportion of abnormal haemoglobin to Hb-A was reduced as compared to that found in the

simple trait. It is difficult to explain this interaction at the level of the gene because the α -thalassaemia locus is not linked to the β -chain structural locus (see Huehns and Shooter, 1965). It is therefore possible that this is due to the preferential formation of Hb-A rather than the abnormal haemoglobin (Weatherall, 1964b). If this is so, then perhaps some Hb- β_4^E , Hb- β_4^C or Hb- β_4^S respectively might be found in these cases. Although Hb- β_4^C and Hb- β_4^S have already been described (Huisman, 1960) they were not found in the cases investigated by Weatherall, neither was Hb- β_4^E by Tuchinda and others (1964).

The Significance of Haemoglobin F after the Postnatal Period

The finding of Hb-F after the postnatal period is not always a sign of thalassaemia. It appears that it can persist in a number of haematological conditions which commence during the neonatal period. The commonest of these are the haemoglobinopathies, but it has also been reported in other congenital haemolytic anaemias (Beaven, Ellis and White, 1960), trisomy 13-15 (Huehns and others, 1964a) and some cases of Down's syndrome (trisomy 21-22) (Weinstein and Rucknagel, 1964). High levels are often found in acute leukaemia in young children. In adult patients, small increases in Hb-F are occasionally found in leukaemia (Beaven and others, 1961), aplastic anaemia (Jonxis, 1963), untreated pernicious anaemia (Beaven and others, 1961), molar pregnancy (Bromberg, Salzberger and Abrahamov, 1957) or during the second trimester of pregnancy (Rucknagel and Chernoff, 1955). Iron deficiency, acquired haemolytic anaemias and congenital heart disease are not associated with a raised Hb-F.

Hereditary Persistence of Foetal Haemoglobin

In this inherited condition there is depression of β - and δ -chain synthesis without haematological abnormality, since there is good compensation by the continued production of Hb-F. In contrast to Hb-F in thalassaemia or sickle cell anaemia, Hb-F is evenly distributed in the cells (Thompson, Mitchener and Huisman, 1961; Bradley, Brawner and Conley, 1961; Fessas and Stamatoyannopoulos, 1964). Several different types have been described.

(i) *African type of Persistent Foetal Haemoglobin* (Thompson and others, 1961). This trait occurs in approximately 1% of Negroes. In the heterozygous form, 20-30% of haemoglobin is Hb-F, the remainder being Hb-A and Hb-A₂.

In the homozygous form (Wheeler and Krevans, 1961) only Hb-F is found. In neither condition is there any significant pathology. The interaction of this trait with β -thalassaemia is called *persistent haemoglobin F—thalassaemia disease*. These patients have the clinical picture of thalassaemia intermedia (Kraus, Koch and Burckett, 1961; Wheeler and Krevans, 1961; Barkhan and Adinolfi, 1962), Hb-F amounting to 65-70%, with a normal level of Hb-A₂. The diagnosis can only be made by family studies.

(ii) *Greek type of Persistent Foetal Haemoglobin* (Fessas and Stamatoyannopoulos, 1964). This trait occurs in about 0.25% of Greeks. In the heterozygous form, 11-18% of Hb-F is found in the red cells, the remainder being Hb-A and Hb-A₂. There are no other haematological abnormalities. This trait has also been described in association with β -thalassaemia, and is known as the *Greek type of persistent Hb-F-thalassaemia disease*. Clinically, it is a form of β -thalassaemia intermedia with 20-40% Hb-F and a raised Hb-A₂.

(iii) A third type of persistence of Hb-F occurs in 1% of the population of Southern Switzerland (Marti, 1963). In this trait, Hb-F is slightly elevated (1-3%); there is no haematological abnormality.

Treatment of Thalassaemia

At present, there is no specific remedy for thalassaemia and the principal forms of treatment available are blood transfusions and splenectomy. As both these have serious disadvantages they should only be used for specific indications. Patients with relatively mild thalassaemia who stabilize with a haemoglobin level above 7.0 g./100 ml. should not receive any treatment except, of course, to tide them over intercurrent illness (Erlandson, Brilliant and Smith, 1964).

In the more severe cases, once it has been decided to transfuse a child regularly, a definite regime should be planned. In some clinics, the indication for transfusion is the appearance of symptoms in the child; in these cases, the haemoglobin may fall to a very low level and there appears to be general agreement that this is not the best criterion. Alternatively, transfusion is recommended whenever the haemoglobin falls to a selected level, and various workers have chosen different values between 7 and 10 g./100 ml. It can be shown theoretically that the amount of blood transfused per year is approximately the same regardless of the level of haemoglobin chosen.

Therefore, there is no advantage to the patient, such as reducing the risk of transfusion siderosis, in choosing the lower level. There are, however, definite advantages to the patient in maintaining the higher level. The patient would feel much better and be able to exert greater physical and mental activity. It has already been shown that children treated in this way develop more rapidly than patients kept on lower haemoglobin levels (Erlandson and others, 1964; Schorr and Radel, 1964; Wolman, 1964). There is also evidence that the deposition of iron in the tissues is not as deleterious as once thought, provided the oxygen supply to the tissues is kept near normal (Wolman, 1964). With the maintenance of high haemoglobin levels, the absorption of iron from the gut might also be less than if anaemia is constantly present. Finally, if high haemoglobin levels are maintained by transfusion the great stimulus to marrow expansion present in disease will disappear, and it might be expected that the gross bone changes usually present will regress or not develop in the first place. In this connection it might be pointed out that fresh blood has a better survival than stored blood and by its use the transfusion requirement may be reduced by 10-20%.

Recently, chelating agents have been used in the treatment of iron overload in thalassaemia (Smith, 1964). Both desferrioxamine and diethylenetriaminepenta acetate (DTPA) were used. Smith suggests that the most effective way to remove iron from heavily iron-laden thalassaemic children is to combine large doses of DTPA at the time of the transfusions with daily injections of desferrioxamine between transfusions. However, the long term efficiency of these drugs in removing iron from the body has not yet been proved, and the possibility of toxic manifestations must be borne in mind (Fairbanks, Watson and Beutler, 1963). It must also be remembered that alimentary iron absorption continues in thalassaemia despite iron overload, and total iron uptake of the body would be reduced if this could be blocked. Investigations on the effect of oral chelating agents on iron absorption suggest that these may only be marginally useful adjuncts to treatment (Bannerman, 1964).

Splenectomy does not improve thalassaemia unless there is hypersplenism usually shown by an increased transfusion requirement or the appearance of thrombocytopenia (Smith, Erlandson, Stern and Schulman, 1960; Bouroncle and Doan, 1964). In these patients, it can be shown that there is considerable

splenic destruction of donor blood with a consequent shortened red cell survival. Following splenectomy, the survival of donor cells often returns to normal. A second indication for splenectomy is gross splenic enlargement such as to cause mechanical interference in the patient's life, with constant discomfort.

In thalassaemia there is an increased demand for folic acid, and several patients with megaloblastic bone marrow changes have been reported. Usually, this is associated with pregnancy (Goldberg and Schwartz, 1954; Jandl and Greenberg, 1959) but may occur at other times (Chanarin, Dacie and Mollin, 1959). There is also some evidence that folic acid deficiency may increase the anaemia of thalassaemia (Luhby and Cooperman, 1961; Luhby, Cooperman, Feldman, Ceraldo, Herrero and Marley, 1961). Such patients should clearly be treated with folic acid. The question arises whether there is subclinical folic acid deficiency in most cases of thalassaemia due to its high utilization by the increased cell division of the bone marrow. Some of the work on folic acid deficiency in sickle cell disease (Watson-Williams, 1965) may be relevant. This author reports four adult patients with normoblastic erythropoietic marrow, severe sexual immaturity and a very rapid plasma clearance of folic acid. All four showed startling improvement in their development on treatment with folic acid. In thalassaemia, the response to treatment has not been so dramatic, but several authors have reported a general improvement and a reduction in transfusion requirement (Luhby and others, 1961). It therefore seems reasonable to give supplements of folic acid to patients with thalassaemia, although startling response to this therapy will be rare.

Iron therapy, particularly parenteral iron, should not be given in thalassaemia unless iron deficiency has been proved by estimation of the serum iron.

Treatment of Haemoglobin H Disease

Since its discovery, Hb-H has been known to be less stable than normal haemoglobin. This leads to intraerythrocytic precipitation of haemoglobin, formation of red cell inclusion bodies (Fig. 5b) and the rapid destruction of the erythrocytes in the spleen at a red cell age of 40-45 days (Rigas and Koler, 1961). The formation of these inclusion bodies and the consequent red cell destruction are considerably increased by oxidation to methaemoglobin. For this reason, the methaemoglobin-inducing drugs (for example, nitrites, sulphonamides and

phenacetin) should be avoided in patients with Hb-H disease. Rigas and Koler (1961) have also shown that after splenectomy the red cell survival is significantly increased to near normal values, with concomitant decrease in anaemia. This beneficial effect of splenectomy has also been reported by other workers (Gouttas and others, 1955; Lie-Injo Luan Eng and Hart, 1963; Woodrow, Noble and Martindale, 1964). Splenectomy should therefore be considered in the more severe cases of Hb-H disease with splenic enlargement, provided splenic destruction of red cells can be demonstrated.

The Distribution of Thalassaemia

The general distribution of thalassaemia in the world is shown in Fig. 7. Very few studies have differentiated between α - and β -thalassaemia. It appears, however, that both occur in the same regions, α -thalassaemia having been reported from Italy, Greece, Israel and other Mediterranean countries, South East Asia, and Africa (see Motulsky, 1964, for references). A general review of the distribution of thalassaemia is given by Allison (1961). (Further references are to be found in the work of the following authors: Lie-Injo Luan Eng, 1964; Flatz, Pik and Sringam, 1965; Malamos and others, 1962; Silvestroni and Bianco, 1963; Motulsky, 1964; Weatherall, 1965).

It is difficult to see why thalassaemia, which, in the homozygous form, is fatal in early life, should be common in such a large part of the population. Haldane (1949) suggested that death from the homozygous disease might be balanced by protection against death from malaria by the trait form (heterosis). A great deal of research to support this idea has been carried out. Comparison of the overall distribution of malaria with that of thalassaemia in the world shows quite good correspondence. However, when the detailed distributions in one country are compared, there are some very serious discrepancies. The details of this fascinating aspect of the thalassaemia story have recently been discussed by Motulsky (1964b).

From the above description, it may appear that thalassaemia only occurs in the areas shown on the map and in individuals originating from them. Recent work has shown that both α -thalassaemia and β -thalassaemia may rarely be found in British or other Northern European individuals (Callender, Mallet and Lehmann, 1961; Buchanan, Kinloch, Hutchison, Pinkerton and Cassidy, 1963; Beaven and others, 1964).

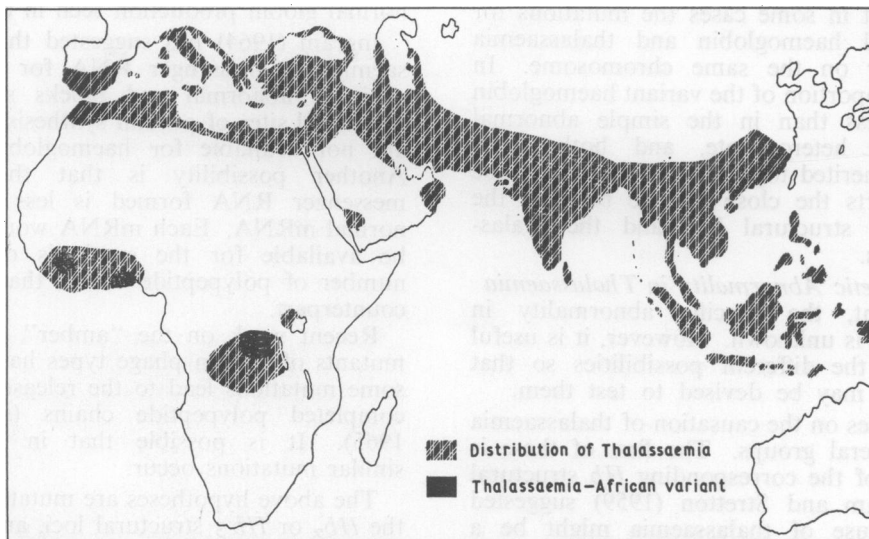


FIG. 7.—Distribution of thalassaemia. (Reprinted with permission from Allison, A. C. (1961), in 'Genetical Variation in Human Populations', ed. Harrison, Pergamon Press Limited).

The Genetic Basis of the Thalassaemia Syndromes

(i) Linkage relationship of the haemoglobin loci and the thalassaemia loci

The loci controlling the structure of the α - and β -chains (Hb_{α} locus and Hb_{β} locus) are not linked. In contrast to this, the Hb_{δ} locus is closely linked to the Hb_{β} locus, no crossovers occurring in at least 45 possibilities. It is also thought that the Hb_{γ} locus is linked to the Hb_{β} + Hb_{δ} complex because the mutation causing persistence of foetal haemoglobin is closely linked or allelic to it, no crossovers occurring in 44 possible instances (for references see Huehns and Shooter, 1965).

Motulsky (1964a) has recently collected the published evidence for linkage between the Hb_{β} locus and the β -thalassaemia locus. Analysis of the offspring of matings of heterozygotes for both β -thalassaemia and a β -chain structural mutant showed that among 80 offspring there were two possible, but not proved, crossovers. These findings indicate that the β -thalassaemia genes are closely linked (or allelic, if the possible crossovers are disproved) to the Hb_{β} structural locus. The close linkage between Hb_{β} and Hb_{δ} loci mentioned above implies that β -thalassaemia is also linked to the latter. There are only relatively few families which throw light on this linkage (Huisman, Punt and Schaad, 1961; Moore and Pearson,

1964), the latter authors reporting one definite crossover. The finding of one crossover (out of 19 possibilities) between the Hb_{δ} locus and β -thalassaemia while there are none (out of 45 possibilities) between the Hb_{β} and Hb_{δ} loci suggests that the Hb_{δ} locus is further from the β -thalassaemia locus than the Hb_{β} locus, i.e. that the β -thalassaemia locus is separate from the Hb_{β} structural locus. Clearly, more family studies are required to resolve this point. The α -thalassaemia locus is not linked to the Hb_{β} locus or the β -thalassaemia locus (Cohen, Zuelzer, Neel and Robinson, 1959; Tuchinda and others, 1964), and this is consistent with the idea that the α -thalassaemia locus is linked to the Hb_{α} locus. However, there are no studies as yet to resolve this point. The biochemical interactions between α -thalassaemia and α -chain mutants of haemoglobin are similar to those between β -thalassaemia and the various β -chain haemoglobin variants, and this suggests that the genetical situation might be analogous to that for β -thalassaemia.

One of the curious features of the thalassaemia genes is that they have not been found in coupling with a haemoglobin mutant. In those cases where the thalassaemia gene completely suppresses the synthesis of the haemoglobin chain concerned there is, of course, no way to determine the nature of the suppressed chain. However, it would be

expected that in some cases the mutations for an abnormal haemoglobin and thalassaemia would occur on the same chromosome. In these, the proportion of the variant haemoglobin would be less than in the simple abnormal haemoglobin heterozygote, and both genes would be inherited together. The lack of these cases supports the close linkage between the haemoglobin structural loci and the thalassaemia genes.

(ii) *The Genetic Abnormality in Thalassaemia*

At present, the specific abnormality in thalassaemia is unknown. However, it is useful to consider the different possibilities so that experiments may be devised to test them.

The theories on the causation of thalassaemia fall into several groups. The first of these is a mutation of the corresponding *Hb* structural locus. Ingram and Stretton (1959) suggested that one cause of thalassaemia might be a hidden amino acid substitution, that is, one that did not result in a difference in electrophoretic mobility of any "haemoglobin A" still being made. This hypothesis could be tested by examining the Hb-A found in cases of Hb-S- β -thalassaemia disease (or other thalassaemia/haemoglobin mutant interactions). Guidotti (unpublished, quoted by Baglioni, 1963) has examined the amino acid composition of the isolated peptides from two such samples of "Hb-A" and found them to be identical with that of the corresponding peptides from Hb-A obtained from a normal adult. Although these findings are strong evidence against this hypothesis, they do not altogether exclude it.

Recently, Itano (1965) has advanced another theory. This idea depends on the degeneracy of the genetic code, that is, the ability of more than one "triplet code word" to code for the same amino acid (see Stretton, 1965). In normal persons, the normal triplet code word is present, but in thalassaemia this is replaced by another which codes for the same amino acid. A hypothetical example would be the change from GGU to GGA, both coding for glycine. One of the factors that limits the rate of addition of any particular amino acid to the growing end of the polypeptide chain during synthesis is the availability of amino acid transfer-RNA fitting any particular coding triplet, and it is argued that there is plenty of transfer-RNA to fit the normal coding triplet but a deficiency of transfer-RNA for the abnormal triplet. This deficiency of availability of an amino acid leads to a block or hold-up at a particular point in the synthesis of the affected polypeptide chain and the decreased

normal globin production seen in thalassaemia.

Ingram (1964) has suggested that in thalassaemia the messenger RNA for the affected chain is abnormal and blocks some of the ribosomal sites of protein synthesis so that they are not available for haemoglobin synthesis. Another possibility is that the abnormal messenger RNA formed is less stable than normal mRNA. Each mRNA would then only be available for the synthesis of a smaller number of polypeptide chains than its normal counterpart.

Recent work on the "amber" and "ochre" mutants of certain phage types has shown that some mutations lead to the release of partially completed polypeptide chains (see Stretton, 1965). It is possible that in thalassaemia similar mutations occur.

The above hypotheses are mutations of either the *Hb α* or *Hb β* structural loci, and proof that the corresponding thalassaemia loci are distinct from these would, of course, rule them out.

The discovery that haemoglobin Lepore (which closely resembles β -thalassaemia in its clinical haematological features) consists of the N-terminal part of the δ -chain joined to the C-terminal part of the β -chain and is probably due to an unequal crossover (Baglioni, 1962, 1965) has led to the speculation that this type of mutation may account for other cases of thalassaemia (Nance, 1963; Smithies, 1964). Although it seems unlikely that this hypothesis is of general application, one situation deserves further discussion. When the amino acid sequences of Hb-A and Hb-A₂ are compared (see Huehns and Shooter, 1965), it can be seen that if the crossover occurs in the first 24 nucleotide pairs (i.e. 8 amino acids), the resulting chain will have the same amino acid sequence as Hb-A. This would appear to fit very well to the finding of Guidotti mentioned above. However, it has to be remembered that during the crossover the *Hb δ* loci are in effect deleted, and heterozygotes should have a low (or normal) Hb-A₂, as is found in Hb-Lepore trait (Gerald and Diamond, 1958b). Thus, the crossover hypothesis would not fit the high A₂ β -thalassaemia but only those cases with a normal Hb-A₂ (the $\beta\delta$ -thalassaemia of Fessas, 1964). If the crossover occurred a little further along the gene, the "Hb-A" formed would contain one or two "silent" amino acid substitutions (at positions 9 and 12), allowing this hypothesis to be tested. If the crossover included position 22 of the δ -chain, of course the electrophoretically separable Hb-Lepore would result.

Finally, it has been suggested that thalassaemia is due to a mutation of a closely linked rate-controlling locus. This hypothesis would, of course, be favoured if a definite crossover between the $Hb\beta$ (or $Hb\alpha$) locus and β (or α)-thalassaemia were discovered. Such a rate-controlling locus has been postulated in the control of bacterial protein synthesis, the operator locus of Jacob and Monod (1961). Although there is no evidence for such regulatory loci in mammals, a number of workers have discussed this concept in relation to thalassaemia (Neel, 1961; Motulsky, 1962; Sturgeon, Schroeder, Jones and Bergren, 1963; Zuckerkandl, 1964). However, these hypotheses do not explain all the findings in thalassaemia.

The multiplicity of hypotheses put forward to explain the findings in thalassaemia indicates that at the present time the data on which a sound theory can be based are not yet available. Finally, it has to be remembered that the thalassaemias are phenotypes which may be caused by different mutations in different individuals.

REFERENCES

- AGER, J. A. M., and LEHMANN, H. (1958): Observations on Some 'Fast' Haemoglobins: K, J, N, and 'Bart's' *Brit. med. J.*, **i**, 929.
- AKSOY, M. (1963): The First Observation of Homozygous Haemoglobin S-alpha-thalassaemia Disease and Two Types of Sick Cell Thalassaemia Disease: a) Sick Cell Alpha-thalassaemia Disease b) Sick Cell Beta-thalassaemia Disease *Blood*, **22**, 757.
- ALLISON, A. C. (1961): Abnormal Haemoglobin and Erythrocyte Enzyme-deficiency Traits. In *Genetical Variation in Human Populations*, ed. G. A. Harrison, p. 16, Oxford: Pergamon Press.
- ASTALDI, G., TOLENTINO, P., and SACCHETTI, C. (1951): La Talassemia (Morbo di Cooley et Forme Affini) Biblioteca "Haematologica" XII, Pavia: Tipografia del Libro.
- ATWATER, J., HUEHNS, E. R., and SHOOTER, E. M. (1961): Haemoglobin I₂, a Further Variant of Haemoglobin A₂, *J. molec. Biol.*, **3**, 707.
- ATWATER, J., SCHWARTZ, I. R., ERSLEV, A. J., MONTGOMERY, T. L., and TOCANTINS, L. M. (1960): Sickling of Erythrocytes in a Patient with Thalassaemia-hemoglobin I Disease, *New Engl. J. Med.*, **263**, 1215.
- BAGLIONI, C. (1962): The Fusion of Two Peptide Chains in Hemoglobin Lepore and its Interpretation as a Genetic Deletion, *Proc. Nat. Acad. Sci. (Wash.)*, **48**, 1880.
- BAGLIONI, C. (1963): Correlations between Genetics and Chemistry of Human Hemoglobins. In *Molecular Genetics*, ed. J. H. Taylor, New York: Academic Press, pt. 1, p. 405.
- BAGLIONI, C. (1964): *Cold Spr. Harb. Symp. quant. Biol.*, **29**, 412.
- BAGLIONI, C. (1965): Abnormal Haemoglobins. X. Chemical Study of Haemoglobin Lepore Boston, *Biochim. biophys. Acta (Amst.)*, **97**, 37.
- BAGLIONI, C., and COLOMBO, B. (1964): Control of Hemoglobin Synthesis, *Cold Spr. Harb. Symp. Quant. Biol.*, **29**, 347.
- BAILEY, I. S., and PRANKERD, T. A. J. (1958): Studies in Thalassaemia, *Brit. J. Haemat.*, **4**, 150.
- BAKER, D. H. (1964): Roentgen Manifestations of Cooley's Anemia, *Ann. N.Y. Acad. Sci.*, **119**, 641.
- BANNERMAN, R. M. (1961): Thalassaemia—A Survey of Some Aspects, New York: Grune and Stratton.
- BANNERMAN, R. M. (1964): Desferrioxamine-B, *Brit. med. J.*, **i**, 304.
- BANNERMAN, R. M., GRINSTEIN, M., and MOORE, C. V. (1959): Haemoglobin Synthesis in Thalassaemia; *in vitro* Studies, *Brit. J. Haemat.*, **5**, 102.
- BARKHAN, P., and ADINOLFI, M. (1962): Observations on the High Foetal Haemoglobin Gene and its Interaction with the Thalassaemia Gene, *J. clin. Path.*, **15**, 350.
- BARNABAS, J., and MULLER, C. J. (1962): Haemoglobin-Lepore Hollandia, *Nature (Lond.)*, **194**, 931.
- BEAVEN, G. H., ELLIS, M. J., and WHITE, J. C. (1960): Studies on Human Foetal Haemoglobin. II. Foetal Haemoglobin Levels in Healthy Children and Adults and in Certain Haematological Disorders, *Brit. J. Haemat.*, **6**, 201.
- BEAVEN, G. H., ELLIS, M. J., and WHITE, J. C. (1961): Studies on Human Foetal Haemoglobin. III. The Hereditary Haemoglobinopathies and Thalassaemia, *Brit. J. Haemat.*, **7**, 169.
- BEAVEN, G. H., STEVENS, B. L., DANCE, N., and WHITE, J. C. (1963): Occurrence of Haemoglobin H in Leukaemia, *Nature (Lond.)*, **199**, 1297.
- BEAVEN, G. H., STEVENS, B. L., ELLIS, M. J., WHITE, J. C., BERNSTOCK, L., MASTERS, P., and STAPLETON, T. (1964): Studies on Human Foetal Haemoglobin. IV. Thalassaemia-like Conditions in British Families, *Brit. J. Haemat.*, **10**, 1.
- BENESH, R. E., RANNEY, H. M., BENESH, R., and SMITH, M. (1961): The Chemistry of the Bohr Effect. II. Some Properties of Haemoglobin H, *J. biol. Chem.*, **236**, 2926.
- BERGREN, W. R., and STURGEON, P. (1960): Haemoglobin H. Some Additional Findings. *Proc. int. Soc. Haemat.*, **7**, (2) part 1, 488.
- BERNINI, L., COLUCCI, C. F., DE MICHELE, D., PIOMELLI, S., and SINISCALCO, M. (1962): A Possible Case of Alpha-beta-thalassaemia, *Acta genet. (Basel)*, **12**, 202.
- BETKE, K., MARTI, H. R., and SCHLICHT, I. (1959): Estimation of Small Percentages of Foetal Haemoglobin, *Nature (Lond.)*, **184**, 1877.
- BINGLE, J. P., HUEHNS, E. R., and PRANKERD, T. A. J. (1958): Haemoglobin-H Disease, *Brit. med. J.*, **ii**, 1389.
- BOURONCLE, B. A., and DOAN, C. A. (1964): Cooley's Anemia: Indications for Splenectomy, *Ann. N.Y. Acad. Sci.*, **119**, 709.
- BRADLEY, T. B. JR., BRAWNER, J. N. III, and CONLEY, C. L. (1961): Further Observations on an Inherited Anomaly Characterized by Persistence of Foetal Haemoglobin, *Bull. Johns Hopk. Hosp.*, **108**, 242.
- BRAIN, M. C., and VELLA, F. (1958): Haemoglobin H Trait in a Nepalese-Gurkha Woman, *Lancet*, **i**, 192.
- BRIDGES, J. M., NEILL, D. W., and LEHMANN, H. (1961): Increase in Hb-A₂ Appearing after Homograft of Foetal Haemopoietic Tissue, *Brit. med. J.*, **i**, 1349.
- BROMBERG, Y. M., SALZBERGER, M., and ABRAHAMOV, A. (1957): Alkali Resistant Type of Hemoglobin in Women with Molar Pregnancy, *Blood*, **12**, 1122.
- BUCHANAN, K. D., KINLOCH, J. D., HUTCHISON, H. E.,

- PINKERTON, P. H., and CASSIDY, P. (1963): Thalassaemia in Scotland, *J. clin. Path.*, **16**, 596.
- BURNHAM, B. F., and LASCELLES, J. (1964): Control of Porphyrin Biosynthesis through a Negative-feedback Mechanism. Studies with Preparations of δ -aminolaevulate Synthetase and δ -aminolaevulate Dehydratase from *Rhodospseudomonas Spheroides*, *Biochem. J.*, **87**, 462.
- CAFFEY, J. (1957): Cooley's Anemia: A Review of the Roentgenographic Findings in the Skeleton, *Amer. J. Roentgenol.*, **78**, 381.
- CALLENDER, S. T., MALLEY, B. J., and LEHMANN, H. (1961): Thalassaemia in Britain, *Brit. J. Haemat.*, **7**, 1.
- CARCASSI, V., CEPPELLINI, R., and PITZUS, F. (1957): Frequenza della Talassemia in Quattro Popolazioni Sarde e Sui Rapporti con la Distribuzione dei Gruppi Sanguigni e della Malaria, *Boll. Ist sieroter. milan.*, **36**, 206.
- CHANARIN, I., DACIE, J. V., and MOLLIN, D. L. (1959): Folic Acid Deficiency in Haemolytic Anaemia, *Brit. J. Haemat.*, **5**, 245.
- CHERNOFF, A. I. (1953): Immunologic Studies of Hemoglobins. II. Quantitative Precipitin Test using Anti-fetal Hemoglobin Sera, *Blood*, **8**, 413.
- CHERNOFF, A. I. (1964): A Method for the Quantitative Determination of HGB A₂, *Ann. N.Y. Acad. Sci.*, **119**, Art. 2, 557.
- CHERNOFF, A. I., PETTIT, N., and NORTHROP, J. (1965): The Amino Acid Composition of Hemoglobin. V. The Preparation of Purified Hemoglobin Fractions by Chromatography on Cellulose Exchanges and the Identification by Starch Gel Electrophoresis using *tris*-borate-E.D.T.A. Buffer, *Blood*, **25**, 646.
- CHOREMIS, C., ZANNOS-MARIOLEA, L., AGER, J. A. M., and LEHMANN, H. (1959): Persistence of Hemoglobin "Bart's" beyond Infancy in a Child with Thalassaemia, *Brit. med. J.*, **ii**, 348.
- COHEN, F., ZUELZER, W. W., NEEL, J. V., and ROBINSON, A. R. (1959): Multiple Inherited Erythrocyte Anomalies in an American Negro Family. Hereditary Spherocytosis, Sickling and Thalassaemia, *Blood*, **14**, 816.
- COOLEY, T. B. (1927): Von Jaksch's Anemia, *Amer. J. Dis. Child.*, **33**, 786.
- COOLEY, T. B., and LEE, P. (1925): A Series of Cases of Splenomegaly in Children with Anemia and Peculiar Bone Changes, *Trans. Amer. pediat. Soc.*, **37**, 29.
- COOLEY, T. B., WITWE, E. R., and LEE, P. (1927): Anemia in Children with Splenomegaly and Peculiar Changes in Bones, Report of Cases, *Amer. J. Dis. Child.*, **34**, 347.
- DACIE, J. V. (1960): The Haemolytic Anaemias, pt. 1. The Congenital Anaemias, London: J. & A. Churchill.
- DANCE, N., HUEHNS, E. R., and BEAVEN, G. H. (1963): The Abnormal Hemoglobins in Hemoglobin-H Disease, *Biochem. J.*, **87**, 240.
- DITTMAN, W. A., HAUT, A., WINTROBE, M. M., and CARTWRIGHT, G. E. (1960): Hemoglobin H Associated with an Uncommon Variant of Thalassaemia Trait, *Blood*, **16**, 975.
- DORMANDY, K. M., LOCK, S. P., and LEHMANN, H. (1961): Hemoglobin Q- α -thalassaemia, *Brit. med. J.*, **i**, 1582.
- ERLANDSON, M. E., BRILLIANT, R., and SMITH, C. H. (1964): Comparison of Sixty-six Patients with Thalassaemia Major and Thirteen Patients with Thalassaemia Intermedia: Including Evaluations of Growth, Development, Maturation and Prognosis, *Ann. N.Y. Acad. Sci.*, **119**, 727.
- ERLANDSON, M. E., SCHULMAN, I., STERN, G., and SMITH, C. H. (1958): Studies on Congenital Hemolytic Syndromes. i) Rates of Destruction and Production of Erythrocytes in Thalassaemia, *Pediatrics*, **22**, 910.
- FAIRBANKS, V. F., WATSON, M. D., and BEUTLER, E. (1963): Drugs for Iron Overload, *Brit. med. J.*, **i**, 1414.
- FESSAS, P. (1960): Observations on a Second Hemoglobin Abnormality in Hemoglobin H Disease, Proc. 7th Congr. Europ. Soc. Haemat., London 1959, Pt. II, 1043, Basel: S. Karger.
- FESSAS, P. (1962): The Beta-chain Thalassaemias. In Hemoglobin Colloquium, ed. H. Lehmann and K. Betke, p. 90, Stuttgart: Thieme.
- FESSAS, P. (1963): Inclusions of Hemoglobin in Erythroblasts and Erythrocytes of Thalassaemia, *Blood*, **21**, 21.
- FESSAS, P. (1964): Forms of Thalassaemia. In Abnormal Hemoglobins in Africa C.I.O.M.S. Symposium on Abnormal Hemoglobins and Enzyme Deficiency, p. 71, Oxford: Blackwell.
- FESSAS, P., and LOUKOPOULOS, D. (1964): Alpha-chain of Human Hemoglobin: Occurrence *in vivo*, *Science*, **143**, 590.
- FESSAS, P., and MASTROKALOS, N. (1959): Demonstration of Small Components in Red Cell Hemolysates by Starch-gel Electrophoresis, *Nature (Lond.)*, **183**, 30.
- FESSAS, P., and PAPASPYROU, A. (1957): New 'Fast' Hemoglobin Associated with Thalassaemia, *Science*, **126**, 1119.
- FESSAS, P., and STAMATOYANNOPOULOS, G. (1962): Absence of Hemoglobin A₂ in an Adult, *Nature (Lond.)*, **195**, 1215.
- FESSAS, P., and STAMATOYANNOPOULOS, G. (1964): Hereditary Persistence of Fetal Hemoglobin in Greece. A Study and a Comparison, *Blood*, **24**, 223.
- FESSAS, P., STAMATOYANNOPOULOS, G., and KARAKLIS, A. (1962): Hemoglobin "Pylos": Study of a Hemoglobinopathy Resembling Thalassaemia in the Heterozygous, Homozygous and Double Heterozygous State, *Blood*, **19**, 1.
- FINK, H. (1964): (Editor), Problems of Cooley's Anemia, *Ann. N.Y. Acad. Sci.*, **119**, Art. 2, 369.
- FLATZ, G., PIK, C., and SRINGAM, S. (1965): Hemoglobin E and β -thalassaemia: their Distribution in Thailand, *Ann. hum. Genet.*, **29**, 151.
- GABUDZA, T. G., NATHAN, D. G., and GARDNER, F. H. (1963): The Turnover of Hemoglobins A, F and A₂ in the Peripheral Blood of Three Patients with Thalassaemia, *J. clin. Invest.*, **42**, 1678.
- GABUDZA, T. G., NATHAN, D. G., and GARDNER, F. H. (1964): Thalassaemia Trait. Genetic Combinations of Increased Fetal and A₂ Hemoglobins, *New Engl. J. Med.*, **270**, 1212.
- GERALD, P. S., and DIAMOND, L. K. (1958a): The Diagnosis of Thalassaemia Trait by Starch Block Electrophoresis of Hemoglobin, *Blood*, **13**, 61.
- GERALD, P. S., and DIAMOND, L. K. (1958b): A New Hereditary Hemoglobinopathy (the Lepore Trait) and its Interaction with Thalassaemia Trait, *Blood*, **13**, 835.
- GOLDBERG, M. A., and SCHWARTZ, S. O. (1954): Mediterranean Anemia in a Negro Complicated by Pernicious Anemia of Pregnancy, *Blood*, **9**, 648.
- GOUTTAS, A. (1962): Les Expressions du Gène Thalassémique en Grèce. In Hemoglobin Col-

- loquium, ed. H. Lehmann and K. Betke, p. 89, Stuttgart: Thieme.
- GOUTTAS, A., FESSAS, P., TSEVRENIS, H., and XEFTERI, E. (1955): Description d'une Nouvelle Variété d'anémie Hémolytique Congénitale: Etude Hématologique Electrophorétique et Génétique, *Sang*, **26**, 911.
- GRINSTEIN, M., BANNERMAN, R. M., VAVRA, J. D., and MOORE, C. V. (1960): Hemoglobin Metabolism in Thalassaemia. *In vivo* Studies, *Amer. J. Med.*, **29**, 18.
- HALDANE, J. B. S. (1949): Disease and Evolution, *La Ricerca Sci. (Suppl.)*, **19**, 68.
- HAMILTON, H. E., SHEETS, R. F., and BROUSSEAU, G. (1962): Gamma Thalassaemia, *J. lab. Clin. Med.*, **60**, 880.
- HENDERSON, A. B., POTTS, E. B., and BURGESS, P. (1962): Sickle Cell Thalassaemia Disease and Pregnancy, *Amer. J. med. Sci.*, **244**, 605.
- HUEHNS, E. R. (1962): Haemoglobin H Disease. Clinical and Experimental Studies, M.D. Thesis, University of London.
- HUEHNS, E. R., BEAVEN, G. H., STEVENS, B. L. (1964): Reaction of Haemoglobin α^A with Haemoglobins β^A_4 , γ^F_4 and δ^A_2 . *Biochem. J.*, **92**, 444.
- HUEHNS, E. R., DANCE, N., BEAVEN, G. H., HECHT, F., and MOTULSKY, A. G. (1964a): Human Embryonic Haemoglobins, *Cold Spr. Harb. Symp. quant. Biol.*, **29**, 327.
- HUEHNS, E. R., DANCE, N., JACOBS, M., BEAVEN, G. H., and SHOOTER, E. M. (1965): Isolation and Properties of the β^A - and γ^F -chain Subunits from Normal Adult and Foetal Haemoglobins, *J. Molec. Biol.*, **12**, 215.
- HUEHNS, E. R., FLYNN, F. V., BUTLER, E. A., and BEAVEN, G. H. (1961): Two New Haemoglobin Variants in a Very Young Human Embryo, *Nature (Lond.)*, **189**, 496.
- HUEHNS, E. R., FLYNN, F. V., BUTLER, E. A., and SHOOTER, E. M. (1960): The Occurrence of Haemoglobin 'Bart's' in Conjunction with Haemoglobin H, *Brit. J. Haemat.*, **6**, 388.
- HUEHNS, E. R., and SHOOTER, E. M. (1962): Reaction of Haemoglobin α^A with Haemoglobin H, *Nature (Lond.)*, **193**, 1083.
- HUEHNS, E. R., and SHOOTER, E. M. (1965): Review article: Human Haemoglobins, *J. Med. Genet.*, **2**, 48.
- HUNT, J. A., and LEHMANN, H. (1959): Haemoglobin 'Bart's': a Foetal Haemoglobin without α -chains, *Nature (Lond.)*, **184**, 872.
- HUISMAN, T. H. J. (1960): Properties and Inheritance of the New Fast Hemoglobin Type found in Umbilical Cord Blood Samples of Negro Babies, *Clin. chim. Acta*, **5**, 709.
- HUISMAN, T. H. J., and DOZY, A. M. (1962): Studies on the Heterogeneity of Hemoglobin. IV. Chromatographic Behavior of Different Human Hemoglobins on Anion-exchange Cellulose (DEAE-cellulose), *J. Chromatog.*, **7**, 180.
- HUISMAN, T. H. J., PUNT, K., and SCHAAD, J. D. G. (1961): Thalassaemia Minor Associated with Hemoglobin B₂ Heterozygosity. A Family Report, *Blood*, **17**, 774.
- HYNES, M., and LEHMANN, H. (1956): Haemoglobin D in a Persian Girl: Presumably the First Case of Haemoglobin-D-thalassaemia, *Brit. med. J.*, **ii**, 923.
- INGRAM, V. M. (1964): A Molecular Model for Thalassaemia, *Ann. N.Y. Acad. Sci.*, **119**, 485.
- INGRAM, V. M., and STRETTON, A. O. W. (1959): Genetic Basis of the Thalassaemia Diseases, *Nature (Lond.)*, **184**, 1903.
- INGRAM, V. M., and STRETTON, A. O. W. (1962a): Human Haemoglobin A₂. I. Comparison of Haemoglobins A₂ and A, *Biochim. biophys. Acta (Amst.)*, **62**, 456.
- INGRAM, V. M., and STRETTON, A. O. W. (1962b): Human Haemoglobin A₂. II. The Chemistry of Some Peptides Peculiar to Haemoglobin A₂, *Biochim. biophys. Acta (Amst.)*, **63**, 20.
- ITANO, H. A. (1965): The Synthesis and Structure of Normal and Abnormal Hemoglobins. In C.I.O.M.S. Symposium on Abnormal Haemoglobins and Enzyme Deficiency, p. 3, Oxford: Blackwell.
- JACOB, F., and MONOD, J. (1961): Genetic Regulatory Mechanisms in the Synthesis of Proteins, *J. Molec. Biol.*, **3**, 318.
- JANDL, J. H., and GREENBERG, M. S. (1959): Bone Marrow Failure due to Relative Nutritional Deficiency in Cooley's Anemia, *New Engl. J. Med.*, **266**, 461.
- JONES, R. T., and SCHROEDER, W. A. (1963): Clinical Characterization and Subunit Hybridization of Human Hemoglobin H and Associated Compounds, *Biochemistry*, **2**, 1357.
- JONES, R. T., SCHROEDER, W. A., BALOG, J. E., and VINOGRAD, J. R. (1959): Gross Structure of Hemoglobin H, *J. Amer. chem. Soc.*, **81**, 3161.
- JONKIS, J. H. P. (1963): Hemoglobinopathies, *Ann. Rev. Med.*, **14**, 297.
- JOSEPHSON, A. N., MASRI, M. S., SINGER, L., DWORKIN, D., and SINGER, K. (1958): Starch Block Electrophoretic Studies of Human Hemoglobin Solutions. II. Results in Cord Blood, Thalassaemia and other Hematologic Disorders: Comparison with Tiselius Electrophoresis, *Blood*, **13**, 543.
- KAPLAN, E., and ZUELZER, W. W. (1950): Erythrocyte Survival Studies in Childhood. II. Studies in Mediterranean Anemia, *J. lab. Clin. Med.*, **36**, 517.
- KARAKLIS, A., and FESSAS, P. (1963): Normal Minor Components of Human Foetal Haemoglobin, *Acta haemat. (Basel)*, **29**, 267.
- KEKWICK, R. A., and LEHMANN, H. (1960): Sedimentation Characteristics of the γ -chain Haemoglobin (Haemoglobin 'Bart's'), *Nature (Lond.)*, **187**, 158.
- KOCHHAR, B. R., and KATHPALIA, P. M. L. (1963): Haemoglobin E-thalassaemia Disease, *Indian J. med. Sci.*, **17**, 138.
- KOLER, R. D., and RIGAS, D. A. (1961): Genetics of Haemoglobin H, *Ann. hum. Genet.*, **25**, 95.
- KRAUS, A. P., KOCH, B., and BURCKETT, L. (1961): Two Families Showing Interaction of Haemoglobin C or Thalassaemia with High Foetal Haemoglobin in Adults, *Brit. med. J.*, **i**, 1434.
- KUNKEL, H. G., CEPPELLINI, R., MULLER-EBERHARD, U. and WOLF, J. (1957): Observations on the Minor Basic Hemoglobin Component in the Blood of Normal Individuals and Patients with Thalassaemia, *J. clin. Invest.*, **36**, 1615.
- KUNKEL, H. G., and WALLENUS, G. (1955): New Hemoglobin in Normal Adult Blood, *Science*, **122**, 288.
- LASCELLES, J. (1960): The Synthesis of Enzymes Concerned in Bacteriochlorophyll Formation in Growing Cultures of *Rhodospseudomonas Spheroides* *J. gen. Microbiol.*, **23**, 487.
- LEE, G. R., HAUT, A., CARTWRIGHT, G. E., and WINTROBE, M. M. (1964): Genetic Control of Hemoglobin A₂ Synthesis: An Unusual Hereditary Pattern in a Family with Thalassaemia Trait, *J. lab. Clin. Med.*, **64**, 72.

- LIE-INJO LUAN ENG (1962): Alpha-chain Thalassemia and Hydrops Fetalis in Malaya. Report of Five Cases, *Blood*, **20**, 581.
- LIE-INJO LUAN ENG (1964): Haemoglobinopathies in East Asia, *Ann. hum. Genet.*, **28**, 101.
- LIE-INJO LUAN ENG, and HART, P. L. DE V. (1963): Splenectomy in Two Cases of Haemoglobin Q-H Disease (Hb-Q- α -thalassaemia), *Acta Haemat. (Basel)*, **29**, 358.
- LONDON, I., BRUNS, G. P., and KARIBIAN, D. (1964): The Regulation of Hemoglobin Synthesis and the Pathogenesis of Some Hypochromic Anemias, *Medicine (Baltimore)*, **43**, 789.
- LUHBY, A. L., and COOPERMAN, J. M. (1961): Folic Acid Deficiency in Thalassemia Major, *Lancet*, **ii**, 490.
- LUHBY, A. L., COOPERMAN, J. M., FELDMAN, P., CERALDO, J., HERRERO, J., and MARLEY, J. F. (1961): Folic Acid as a Limiting Factor in the Anemias of Thalassemia Major, *Blood*, **18**, 786.
- MALAMOS, B., BELCHER, E. H., GYFTAKI, E., and BINOPOULOS, D. (1961): Simultaneous Studies with Fe⁵⁹ and Cr⁵¹ in Congenital Haemolytic Anaemia, *Nucl. Med. (Stuttg.)*, **2**, 1.
- MALAMOS, B., FESSAS, P., and STAMATOYANNOPOULOS, G. (1962): Types of Thalassemia-trait Carriers as Revealed by a Study of their Incidence in Greece, *Brit. J. Haemat.*, **8**, 5.
- MARKS, P. A., and BURKA, E. R. (1964): Hemoglobins A and F: Formation in Thalassemia and Other Hemolytic Anemias, *Science*, **144**, 552.
- MARTI, H. R. (1963): Normale und Anomale Menschliche Hämoglobine, p. 85, Berlin: Springer.
- MONTI, A., FELDHAKE, C., and SCHWARTZ, S. O. (1964): The S-thalassemia Syndrome, *Ann. N.Y. Acad. Sci.*, **119**, 474.
- MOORE, M. M., and PEARSON, H. A. (1964): Human Hemoglobin Gene Linkage with an Apparent Crossing Over between Thalassemia and Delta Loci, *Clin. Res.*, **12**, 34.
- MOSELY, J. E. (1962): The Thalassemias. Variants and Roentgen Bone Changes, *J. Mt. Sinai Hosp.*, **29**, 199.
- MOTULSKY, A. G. (1962): Controller Genes in Synthesis of Human Haemoglobin, *Nature (Lond.)*, **194**, 607.
- MOTULSKY, A. G. (1964a): Current Concepts of the Genetics of Thalassemia, *Cold Spr. Harb. Symp. quant. Biol.*, **29**, 399.
- MOTULSKY, A. G. (1964b): Hereditary Red Cell Traits and Malaria, *Amer. J. trop. Med. Hyg.*, **13**, 147.
- NA-NAKORN, S. (1957): Studies on Hemoglobin E. III. Homozygous Hemoglobin E and Variants of Thalassemia and Hemoglobin E. A Family Study, *Blood*, **12**, 529.
- NANCE, W. E. (1963): Genetic Control of Hemoglobin Synthesis, *Science*, **141**, 123.
- NEEB, H., BEIBOER, J. L., JONXIS, J. H. P., KAARS SJPSTEIJN, J. A., and MULLER, C. J. (1961): Homozygous Lepore Haemoglobin Disease Appearing as Thalassemia Major in Two Papuan Siblings, *Trop. geogr. Med.*, **13**, 207.
- NEEL, J. V. (1961): The Hemoglobin Genes: A Remarkable Example of the Clustering of Related Functions on a Single Mammalian Chromosome, *Blood*, **18**, 769.
- PEARSON, H. A. (1964): Thalassemia Intermedia Genetic and Biochemical Considerations, *Ann. N.Y. Acad. Sci.*, **119**, 369.
- PEARSON, H. A., GERALD, P. S., and DIAMOND, L. K. (1959): Thalassemia Intermedia due to Interaction of Lepore Trait with Thalassemia, *Amer. J. Dis. Child.*, **97**, 464.
- RAMOT, B., SHEBA, C., FISHER, S., AGER, J. A. M., and LEHMANN, H. (1959): Haemoglobin H Disease with Persistent Haemoglobin Bart's in an Oriental Jewess and her Daughter. A Dual Alpha-chain Deficiency of Human Haemoglobin, *Brit. med. J.*, **ii**, 1228.
- RIGAS, D. A., and KOLER, R. D. (1961): Decreased Erythrocyte Survival in Hemoglobin H Disease as a Result of the Abnormal Properties of Hemoglobin H. The Benefit of Splenectomy, *Blood*, **18**, 1.
- RIGAS, D. A., KOLER, R. D., and OSGOOD, E. E. (1955): New Hemoglobin Possessing a Higher Electrophoretic Mobility than Normal Adult Hemoglobin, *Science*, **121**, 372.
- RIGAS, D. A., KOLER, R. D., and OSGOOD, E. E. (1956): Hemoglobin H; Clinical, Laboratory, and Genetic Studies of a Family with a Previously Undescribed Hemoglobin, *J. lab. Clin. Med.*, **47**, 51.
- RIMINGTON, C. (1959): Biosynthesis of Haemoglobin, *Brit. med. Bull.*, **15**, 19.
- RUCKNAGEL, D. L., and CHERNOFF, A. I. (1955): Immunologic Studies of Hemoglobins. III. Fetal Hemoglobin Changes in the Circulation of Pregnant Women, *Blood*, **10**, 1092.
- SCHORR, J. B., and RADEL, E. (1964): Transfusion Therapy and its Complications in Patients with Cooley's Anemia, *Ann. N.Y. Acad. Sci.*, **119**, 703.
- SCHROEDER, W. A. (1963): The Hemoglobins, *Ann. Rev. Biochem.*, **32**, 301.
- SHEPARD, M. K., WEATHERALL, D. J., and CONLEY, C. L. (1962): Semi-quantitative Estimations of the Distribution of Fetal Hemoglobin in Red Cell Populations, *Bull. Johns Hopk. Hosp.*, **110**, 293.
- SILVESTRONI, E., and BIANCO, I. (1957): Sull'esistenza nella Popolazione Italiana di Soggetti non Microcitemici Portatori di una Elevata Quota di Emoglobina Adulta Lenta (Hb-A₂), *Policlinico, Sez. Prat.*, **64**, 1868.
- SILVESTRONI, E., and BIANCO, I. (1958): Associazione di Hb.G e Microcitemia in due Membri di una Famiglia Italiana del Ferrarese, *Policlinico, Sez. Prat.*, **55**, 203.
- SILVESTRONI, E., and BIANCO, I. (1963a): Le Emoglobine Umane, Rome: Edizioni Dell'istituto "Gregorio Mendel".
- SILVESTRONI, E., and BIANCO, I. (1963b): Abnormal Haemoglobin in Italy, *Ann. Soc. belge Med. trop.*, **4**, 683.
- SILVESTRONI, E., BIANCO, I., MUZZOLINI, M., MODIANO, G., and VALLESNERI, E. (1957): Dell'emoglobina di Malati di Anemia Microcitica Costituzione e di Morbo di Cooley, *Progr. Med.*, **13**, 705.
- SINGER, K., CHERNOFF, A. I., and SINGER, L. (1951): Studies on Abnormal Hemoglobins. I. Their Demonstration in Sickle-cell Anemia and other Hematologic Disorders by Means of Alkali Denaturation, *Blood*, **6**, 413.
- SINGER, K., and FISHER, B. (1952): Studies on Abnormal Hemoglobins. V. The Distribution of Type S (sickle cell) Hemoglobin and Type F (Alkali Resistant) Hemoglobin within the Red Cell Population in Sickle Cell Anemia, *Blood*, **7**, 1216.
- SINGER, K., KRAUS, A. P., SINGER, L., RUBINSTEIN, H. M., and GOLDBERG, S. R. (1954): A New Syndrome: Hemoglobin C-thalassemia Disease, *Blood*, **9**, 1032; **12**, 593.
- SINGER, K., SINGER, L., and GOLDBERG, S. R. (1955): Studies on Abnormal Hemoglobins. XI. Sickle Cell-thalassemia Disease in the Negro. The Significance

- of the S+A+F and S+A Patterns Obtained by Hemoglobin Analysis, *Blood*, **10**, 405.
- SMITH, C. H., ERLANDSON, M. E., STERN, G., and SCHULMAN, I. (1960): The Role of Splenectomy in the Management of Thalassaemia, *Blood*, **15**, 197.
- SMITH, R. S. (1964): Chelating Agents in the Diagnosis and Treatment of Iron Overload in Thalassaemia, *Ann. N.Y. Acad. Sci.*, **119**, 776.
- SMITHIES, O. (1964): Chromosomal Rearrangement and Protein Structure, *Cold Spr. Harb. Symp. quant. Biol.*, **29**, 309.
- STEINER, M., BALDINI, M., DAMESHEK, W. (1964): Enzymatic Defects of Heme Synthesis in Thalassaemia, *Ann. N.Y. Acad. Sci.*, **119**, 548.
- STRETTON, A. O. W. (1965): The Genetic Code, *Brit. med. Bull.*, **21**, 229.
- STURGEON, P., and FINCH, C. A. (1957): Erythrokinetics in Cooley's Anemia, *Blood*, **12**, 64.
- STURGEON, P., SCHROEDER, W. A., JONES, R. T., and BERGREN, W. R. (1963): The Relation of Alkali-resistant Haemoglobin in Thalassaemia and Abnormal Haemoglobin Syndromes to Foetal Haemoglobin, *Brit. J. Haemat.*, **9**, 438.
- SYDENSTRYCKER, U. P., HORTON, B., PAYNE, R. A., and HUISMAN, T. H. J. (1961): Studies on a Fast Haemoglobin Variant Found in a Negro Family in Association with Thalassaemia, *Clin. chim. Acta*, **6**, 677.
- THOMPSON, R. B., MITCHENER, J. W., and HUISMAN, T. H. J. (1961): Studies on the Foetal Hemoglobin in the Persistent High Hb-F Anomaly, *Blood*, **18**, 267.
- TUCHINDA, S., RUCKNAGEL, D. L., MINNICH, V., BOONYAPRAKOB, U., BALANKURA, K., and SUVATEE, V. (1964): The Coexistence of Genes of Hemoglobin E and α -thalassaemia in Thais, with Resultant Suppression of Hemoglobin E Synthesis, *Amer. J. hum. Genet.*, **16**, 311.
- VELLA, F., WELLS, R. H. C., AGER, J. A. M., and LEHMANN, H. (1958): A Haemoglobinopathy Involving Haemoglobin H and a New (Q) Haemoglobin, *Brit. med. J.*, **i**, 752.
- VENTRUTO, V., BAGLIONI, C., ROSA, L. DE, BIANCHI, P., COLOMBO, B., and QUATTRIN, N. (1965): Haemoglobin Caserta: an Abnormal Haemoglobin Observed in a Southern Italian Family, *Scand. J. Haemat.*, **2**, 118.
- WARRINGTON, R. L., BELL, W. N., and THOMPSON, R. B. (1965): Interaction between Persistent Hb-F Gene and Delta Chain Type of Thalassaemia, *Clin. Res.*, **13**, 38.
- WASI, P., NA-NAKORN, S., and SVINGDAMRONG, A. (1964): Thalassaemia-Hb-H in Thailand: a Genetical Study, *Proc. Xth Congr. Int. Soc. Haemat.*, *in press*.
- WATSON-WILLIAMS, E. J. (1965): The Role of Folic Acid in the Treatment of Sickle Cell Disease. C.I.O.M.S. Symposium on Abnormal Haemoglobins in Africa, ed. J. H. P. Jonxis, p. 435, Oxford: Blackwell.
- WEATHERALL, D. J. (1963): Abnormal Haemoglobins in the Neonatal Period and their Relationships to Thalassaemia, *Brit. J. Haemat.*, **9**, 265.
- WEATHERALL, D. J. (1964a): Biochemical Phenotypes of Thalassaemia in the American Negro Population. Problems in Cooley's Anemia, *Ann. N.Y. Acad. Sci.*, **119**, 450.
- WEATHERALL, D. J. (1964b): The Relationship of Hemoglobins Bart's and H to α -thalassaemia. Problems in Cooley's Anemia, *Ann. N.Y. Acad. Sci.*, **119**, 463.
- WEATHERALL, D. J. (1965): The Thalassaemia Syndromes, Oxford: Blackwell.
- WEINSTEIN, E. D., and RUCKNAGEL, D. L. (1965): Quantitative Studies on A₂, Sickle Cell and Foetal Hemoglobins in Negroes with Down's Syndrome (Mongolism), *Amer. J. hum. Genet.*, *in press*.
- WHEELER, J. T., and KREVANS, J. R. (1961): The Homozygous State of Persistent Foetal Hemoglobin and the Interaction of Persistent Foetal Hemoglobin with Thalassaemia, *Bull. Johns Hopk. Hosp.*, **109**, 215.
- WHITE, J. C., ELLIS, M., COLEMAN, P. N., BEAVEN, G. H., GRATZER, W. B., SHOOTER, E. M., and SKINNER, E. R. (1960): An Unstable Haemoglobin Associated with Some Cases of Leukaemia. *Brit. J. Haemat.*, **6**, 171.
- WINTROBE, M. M. (1963): Clinical Haematology, 5th ed., London: Henry Kimpton.
- WOLF, J. A., and IGNATOV, V. G. (1963): Heterogeneity of Thalassaemia Major, *Amer. J. Dis. Child.*, **105**, 234.
- WOLMAN, I. (1964): Transfusion Therapy in Cooley's Anemia: Growth and Health as Related to Long-range Hemoglobin Levels. A Progress Report, *Ann. N.Y. Acad. Sci.*, **119**, 736.
- WOODROW, J. C., NOBLE, R. L., and MARTINDALE, J. H. (1964): Haemoglobin H Disease in an English Family, *Brit. med. J.*, **i**, 36.
- ZUCKERKANDL, E. (1964): Controller-gene Diseases. The Operon Model Applied to β -thalassaemia, Familial Foetal Hemoglobinemia and the Normal Switch from the Production of Foetal Hemoglobin to that of Adult Hemoglobin, *J. Molec. Biol.*, **8**, 128.
- ZUELZER, W. W., and KAPLAN, E. (1954): Thalassaemia-hemoglobin C Disease, *Blood*, **9**, 1047.
- ZUELZER, W. W., NEEL, J. V., and ROBINSON, A. R. (1956): Abnormal Hemoglobins. *In Progress in Hematology*, ed. L. M. Tocantins, p. 91. New York: Grune and Stratton.
- ZUELZER, W. W., ROBINSON, A. R., and BOOKER, C. R. (1961): Reciprocal Relationship of Hemoglobins A₂ and F in Beta-chain Thalassaemias, a Key to the Genetic Control of Hemoglobin F. *Blood*, **17**, 393.